Appeal No. 2017-1010

United States Court of Appeals
for the
Federal Circuit

AMGEN INC., AMGEN MANUFACTURING LIMITED,

Plaintiffs-Appellants,

– v. –

APOTEX INC., APOTEX CORP.,

Defendants-Appellees.

APPEAL FROM THE UNITED STATES DISTRICT COURT FOR THE SOUTHERN DISTRICT OF FLORIDA IN CASE NO. 0:15-CV-61631-JIC (CONSOLIDATED WITH 0:15-CV-62081-JIC), JUDGE JAMES I. COHN

BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING LIMITED

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December 5, 2016

CERTIFICATE OF INTEREST

1. The full name of every party represented by me is:

   AMGEN INC. and AMGEN MANUFACTURING LIMITED

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

   AMGEN INC. and AMGEN MANUFACTURING LIMITED

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party represented by me are:

   AMGEN INC.

4. The names of all law firms and the partners and associates that appeared for the party now represented by me in the trial court or are expected to appear in this Court (and who have not or will not enter an appearance in this case) are:

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STATEMENT OF RELATED CASES

This Court (Judges Wallach, Bryson, and Taranto) previously considered Apotex's appeal of the district court's grant of a preliminary injunction in the same action at issue in this appeal. *See Amgen Inc. v. Apotex Inc.*, 827 F.3d 1052 (Fed. Cir. 2016) (No. 16-1308). This Court issued an opinion on July 5, 2016, the mandate issued on August 11, 2016, and Apotex then petitioned the Supreme Court for a writ of certiorari. *See Apotex Inc. v. Amgen Inc.*, No. 16-332.

U.S. Patent No. 8,952,138 is currently the subject of a petition for *inter partes* review: *Apotex Inc. v. Amgen Inc.*, IPR2016-01542 (filed Aug. 5, 2016). As of the filing of this brief, the Patent Trial and Appeal Board has yet to determine whether to institute trial. No other related cases are known to counsel for Amgen to be pending in this or any other court that will directly affect or be affected by this Court's decision on appeal.

STATEMENT OF JURISDICTION

The district court has subject-matter jurisdiction over this case under 28 U.S.C. §§ 1331 and 1338(a). This Court has jurisdiction over this appeal under 28 U.S.C. § 1295(a)(1). Amgen timely appealed under 28 U.S.C. § 2107 and Fed. R. App. P. 4(a) on October 3, 2016. (*See* Appx66-68.)

STATEMENT OF THE ISSUES

1. Where the plain language of claim 1 of U.S. Patent No. 8,952,138 (the "'138 Patent") recites that the redox buffer strength has a value of "2 mM or greater," did the district court erroneously construe this phrase to mean "2 mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100 mM," where such construction imported limitations into the claims based on disclosure that pertained only to specific embodiments of the invention and where neither the specification nor prosecution history otherwise provided a basis for imposing an effective upper limit?

2. According to claim 1 of the '138 Patent, the "redox buffer strength of 2 mM or greater" is associated with a "redox component," comprising oxidants and reductants, which become diluted when part of the "refold mixture." Assuming *arguendo* that the district court correctly construed "2 mM or greater" to be effectively capped at 100 mM, did it nonetheless clearly err in finding that the corresponding redox component of Apotex's process did not infringe equivalently, even though, upon dilution in Apotex's refold mixture, it delivers the same amount of oxidants and reductants as the claimed process and otherwise provides refold conditions insubstantially different from those within the scope of the claim?

3. The district court construed the refold mixture of claim 1 of the '138 Patent to have a protein concentration "at or above about 1 g/L protein." Did the district

court err in finding that Apotex's process did not satisfy this claim term for the following reasons:

a. By declining to accord any probative value to factual statements made by Apotex in its statements pursuant to subparagraph 262(*l*)(3)(B) of the Biologics Price Competition and Innovation Act of 2009 ("BPCIA");

b. By declining to recognize, as part of claim construction, that the patent equates protein and washed inclusion bodies; or

c. By failing to base its infringement analysis on the specifications of Apotex's abbreviated Biologics License Applications ("aBLAs")?

STATEMENT OF THE CASE

Apotex seeks FDA approval to make and sell biosimilar versions of Amgen's NEULASTA® (pegfilgrastim) and NEUPOGEN® (filgrastim). (Appx13-14.) FDA has not yet approved those applications. (*See id.*) This is an appeal from a consolidated patent-infringement case brought by Amgen against Apotex pursuant to paragraph 262(*l*)(6) of the BPCIA. (Appx13-14.) *See* 42 U.S.C. § 262(*l*)(6); 35 U.S.C. § 271(e)(2)(C).

STATEMENT OF THE FACTS

A. Amgen's NEULASTA® and NEUPOGEN® Products

Amgen Inc. discovers, develops, manufactures, and sells innovative therapeutic products based on advances in molecular biology, recombinant DNA technology, and chemistry. (Appx151-169 at Appx151-152; Appx170-191 at Appx170.) Amgen Manufacturing Limited manufactures and sells biologic medicines for treating human diseases. (Appx151-152; Appx170.)

Amgen's NEULASTA® (pegfilgrastim) and NEUPOGEN® (filgrastim) are recombinantly produced proteins that stimulate the production of neutrophils, a type of white blood cell. (Appx1-12 at Appx1-2.) One of their uses is to counteract neutropenia, a neutrophil deficiency that makes a person highly susceptible to life-threatening infections and is a common side effect of certain chemotherapeutic drugs. (Appx158-159; Appx177-178.)

NEULASTA® and NEUPOGEN® were approved under the traditional biologics regulatory pathway, 42 U.S.C. § 262(a). (*See* Appx160; Appx179; Appx243-245.) Amgen demonstrated to FDA that NEULASTA® and NEUPOGEN® are "safe, pure, and potent." 42 U.S.C. § 262(a)(2)(C)(i)(I). Amgen Inc. owns the FDA licenses for NEULASTA® and NEUPOGEN®. (*See* Appx1; Appx13-42 at Appx13-14; Appx596-604 at Appx598.)

B. Apotex's Biosimilar Pegfilgrastim and Filgrastim

Apotex Inc. develops, manufactures, and sells pharmaceuticals, including generic medicines. (Appx151-152; Appx170-171.) Apotex Corp. markets those pharmaceuticals in the United States. (Appx151-152; Appx170-171.)

Apotex filed aBLAs under the BPCIA's abbreviated pathway, 42 U.S.C. § 262(k), seeking approval of its biosimilar pegfilgrastim and filgrastim products, designating Amgen's NEULASTA® and NEUPOGEN®, respectively, as the reference products. (Appx13-14.)

C. The Parties' Exchanges of Information Pursuant to the BPCIA

The BPCIA "established a unique and elaborate process for information exchange between the biosimilar applicant and the [Reference Product Sponsor (or, 'Sponsor')] to resolve patent disputes." *Amgen Inc. v. Sandoz Inc.*, 794 F.3d 1347, 1352 (Fed. Cir. 2015), *petitions for cert. filed*, No. 15-1039, No. 15-1195.

Apotex and Amgen engaged in the information exchanges described in paragraph 262(*l*)(3) for both Apotex's biosimilar pegfilgrastim and filgrastim products. (Appx160-161; Appx179-180.) Pursuant to subparagraph 262(*l*)(2)(A), Apotex provided Amgen with its aBLAs for pegfilgrastim and filgrastim. (Appx160; Appx179.) Amgen then identified patents for which it "believe[d] a claim of patent infringement could reasonably be asserted," pursuant to subparagraph 262(*l*)(3)(A). (Appx160; Appx179.) Amgen listed, inter alia, the

’138 Patent. (*See* Appx152-153; Appx171-172.) Pursuant to subparagraph 262(*l*)(3)(B), Apotex provided Amgen with “a detailed statement that describes, on a claim by claim basis, the factual and legal basis of its opinion” that the ’138 Patent “is invalid, unenforceable, or will not be infringed by the commercial marketing” of Apotex’s biosimilar products. (Appx7382; Appx7427.) Pursuant to subparagraph 262(*l*)(3)(C), Amgen then provided Apotex a “detailed statement” describing “the factual and legal basis” of its opinion that the ’138 Patent will be infringed by the commercial marketing of Apotex’s biosimilar products. (*See* Appx11215-11216; Appx11250-11251.) In both of its subparagraph 262(*l*)(3)(C) statements, Amgen cited to and relied on the factual information in Apotex’s subparagraph 262(*l*)(3)(B) statements. (*See* Appx11225; Appx11269.)

For each product, after these exchanges, pursuant to paragraph 262(*l*)(4), Apotex and Amgen agreed that Amgen would file suit under paragraph 262(*l*)(6) on, inter alia, the ’138 Patent. (Appx153; Appx172.) Amgen accordingly asserted infringement under 35 U.S.C. § 271(e)(2)(C) and sought a declaratory judgment of infringement under 35 U.S.C. § 271(g). (Appx162-165; Appx181-183.) Apotex counterclaimed for a declaratory judgment of non-infringement. (Appx376-378; Appx510-511.) The pegfilgrastim and filgrastim lawsuits were then consolidated. (Appx412-413.)

D. The ’138 Patent

The ’138 Patent is directed to improved methods of refolding proteins expressed in non-mammalian (e.g., bacterial) cell-culture systems, using redox chemicals and other additives to achieve refolding at high protein concentrations. (Appx48-65.)

The ’138 Patent addresses a problem associated with producing recombinant proteins in non-mammalian cells at commercial scale. (Appx57.) Bacterial cells typically are unable to produce such proteins as properly folded molecules, especially at high expression levels. (*Id.*) The misfolded proteins precipitate within the bacterial cells in aggregates called “inclusion bodies.” (*Id.*) The inclusion bodies must be isolated and solubilized so that the incorrectly folded proteins are unfolded and subsequently refolded to form the proper three-dimensional conformation. (*Id.*) Proper folding is necessary for the protein to have biological activity. (Appx57, Appx60.)

When a protein contains the amino-acid cysteine, as most do, each cysteine residue is capable of forming a disulfide bond with another cysteine residue. (Appx57, Appx62.) The formation of certain disulfide bonds between cysteine residues of a protein are part of “proper” or “correct” protein folding. (Appx57; *see* Appx9808.) However, other disulfide bonds may form between cysteine residues of the same protein molecule, leading to “improper” or “incorrect” folding

(and, usually, lack of biological activity). (Appx57; *see* Appx9808.) Similarly, disulfide bonds may form between cysteine residues of different protein molecules, leading to protein aggregation, another undesirable result. (Appx57.)

Prior means of refolding recombinant proteins generally used very dilute protein solutions, which necessitated the use of very large volumes for commercial scale. (Appx57.) Under such conditions, which allow for the protein molecules to be spatially separated, the risk of protein aggregation decreases, so that a protein can properly fold on itself. (Appx57; *see* Appx9817-9818.)

The inventors of the '138 Patent invented an improved method for refolding proteins. They uniquely appreciated specific relationships between the redox buffer strength, thiol-pair ratio, and protein concentration. (Appx60-61.) Their patent teaches how to create optimal refolding environments by applying defined equations for redox buffer strength and thiol-pair ratio. (*Id.*) These teachings optimize and enhance the refolding of proteins at concentrations significantly higher (less dilute) than typical in the prior art, which can reduce the practical and economic problems associated with refolding proteins in physically large volumes. (Appx57.)

E. The District Court's April 7, 2016 Claim Construction

Claim 1, the sole independent claim of the '138 Patent, reads:

> 1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:
> (a) contacting the protein with a refold buffer comprising
> a redox component comprising
> a final thiol-pair ratio having a range of 0.001 to 100 and
> a redox buffer strength of 2 mM or greater
> and one or more of:
> (i) a denaturant;
> (ii) an aggregation suppressor; and
> (iii) a protein stabilizer;
> to form a refold mixture;
> (b) incubating the refold mixture; and
> (c) isolating the protein from the refold mixture.

(Appx65.)

The claim requires contacting a protein-containing "volume" with a "refold buffer" to form a "refold mixture." (Appx65; *see* Appx9832.) The "refold buffer" comprises, inter alia, a "redox component" having a "redox buffer strength" of "2 mM or greater." (Appx65.)

The district court construed the term "2 mM or greater" to mean "2mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100mM." (Appx10.) The court agreed with Apotex that, although the claim specifies no upper boundary, the redox buffer strength should be effectively bounded at 100 mM. (Appx9-10.)

For "refold mixture," Apotex argued its protein concentration must be 2 g/L or greater. (Appx9.) Amgen disagreed. Focusing on the liquid to which the "2 g/L or greater" limitation applies, Amgen argued that 2 g/L is the lowest protein

concentration in the protein-containing "volume" before it is contacted with the "refold buffer" to form the "refold mixture"; therefore, because the protein-containing "volume" is necessarily diluted with the "refold buffer" to form the "refold mixture," the protein concentration in the "refold mixture" could be lower than 2 g/L. (*Id.*) Amgen argued that the "refold mixture" should be construed to have a minimum protein concentration of about 1 g/L. (*Id.*) Amgen cited to both the patent specification, which specifies that the dilution could result in a protein concentration as low as 1 g/L, and extrinsic evidence showing that high protein-concentration refolding starts at about 1 g/L. (Appx966-967; *see* Appx62.) The district court agreed with Amgen and construed "refold mixture" to mean "a mixture formed from contacting (1) the volume in which the concentration of protein is 2.0 g/L or greater with (2) the refold buffer. The refold mixture has a high protein concentration, where 'high protein concentration' is at or above about 1g/L protein." (Appx9.) The court found that the "language of the claim, the specification and the state of the prior art support the conclusion that the refold mixture" would be interpreted by a skilled artisan to have a minimum protein concentration of about 1 g/L. (Appx9.)

F. The Evolution of Apotex's Protein Concentration Positions

Over the course of the litigation, including during its rebuttal case at trial, Apotex took three evolving positions regarding the protein concentration in its

refold mixture. First, in its statements pursuant to subparagraph 262(*l*)(3)(B) of the BPCIA, Apotex twice asserted that its refold mixture has 0.9-1.4 g/L of filgrastim (the protein of interest), without specifying the concentration of other proteins in the refold mixture. (Appx7396; Appx7447). Second, Apotex asserted that its refold mixture has 0.9-1.4 g/L of total protein, with no more than 0.53 g/L filgrastim. Third, in its rebuttal case at trial, Apotex for the first time asserted that its refold mixture has an inclusion-body concentration of 0.9-1.4 g/L, about two-thirds being water and one-third being protein, leaving a total protein concentration of about 0.3-0.5 g/L, and, if measured by chromatography, has a maximum total protein concentration of 0.708 g/L.

In its pre-litigation BPCIA disclosures pursuant to subparagraph 262(*l*)(3)(B), Apotex contended that claim 1 required a protein concentration of 2 g/L or greater in the "refold mixture," and represented to Amgen that it did not infringe the '138 Patent because the filgrastim concentration of Apotex's refold mixture was 0.9-1.4 g/L. (Appx7396; Appx7447.) Apotex cited its aBLA specification that the washed-inclusion-body concentration of Apotex's refold mixture was 0.9-1.4 g/L. (Appx7396; Appx7447.) This protein concentration—twice asserted by Apotex in its subparagraph 262(*l*)(3)(B) statements—falls within the protein-concentration limitation of the "refold mixture" of claim 1, as ultimately construed by the district court, i.e., "at or above about 1 g/L." (Appx9.)

Amgen relied on this in its subparagraph 262(*l*)(3)(C) statements: "As Apotex notes at page 15 of its (3)(B) Statement, 'the concentration of its filgrastim critical intermediate in the refold buffer is 0.9-1.4 g/L.'" (Appx11225; *accord* Appx11269.)

After the district court issued its claim-construction order, Apotex changed its position with respect to the protein concentration in its refold mixture. Apotex's expert Dr. Robinson disagreed with Amgen's expert Dr. Willson that the protein concentration in Apotex's refold mixture is 0.9-1.4 g/L." (Appx10372.) She opined that the filgrastim concentration in Apotex's refold mixture is 0.19-0.53 g/L. (Appx10373.)

At her April 21, 2016 deposition, Dr. Robinson also stated that the total protein concentration—as opposed to the filgrastim concentration—in Apotex's refold mixture is the 0.9-1.4 g/L inclusion-body concentration. (Appx9606; *see* Appx3517-3519.) She confirmed that any non-proteinaceous components of Apotex's inclusion bodies could be "membranes, cell membranes, lipids"—not water. (Appx9605; *see* Appx3525.) None of this testimony was changed in Dr. Robinson's deposition errata. (Appx9620-9622; *see* Appx3519-3520.) Further, Dr. Robinson did not alter any of this testimony during her later July 8, 2016 deposition, three days before trial. (*See* Appx11123.)

Following the close of expert discovery, Apotex moved for summary judgment arguing that its refold mixture has a "protein" concentration less than about 1 g/L. (Appx1609.) Since the term "protein" had not been interpreted during the *Markman* phase of the case, Apotex took the position that "protein" meant "protein of interest" and argued that the "protein" concentration of its refold mixture never exceeds the 0.53 g/L filgrastim concentration discussed in Dr. Robinson's expert report. (Appx1600, Appx1609-1611.)

Amgen opposed. Although disagreeing with Apotex's assertion that 0.53 g/L is the maximum filgrastim concentration, Amgen explained that the total protein concentration in Apotex's refold mixture falls within claim 1 because (1) the "protein" of claim 1 refers to the total protein in the refold mixture, not just the protein of interest (Appx1751-1753), and (2) both experts agreed that the total protein concentration is 0.9-1.4 g/L (Appx1748-1755).

Finding that the parties "now dispute the meaning of the term 'protein,'" i.e., total protein (Amgen) versus protein of interest (Apotex), the district court ordered supplemental claim-construction briefing. (Appx2326; Appx2466-2467.) Subsequently, in an Omnibus Order, the district court construed the term "protein" to mean "'any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds,' including but not limited to the protein of interest." (Appx2453-2472 at Appx2468 (emphasis added).) The court also denied Apotex's

summary-judgment motion, finding that under its construction of "protein," a reasonable factfinder could conclude that Apotex's process satisfies the claim limitation of "refold mixture" having a "high protein concentration" of "at or above about 1 g/L protein." (*See* Appx2471.) The court based its decision in part on Dr. Robinson's testimony that the total protein amount in Apotex's refold mixture is the 0.9-1.4 g/L inclusion-body concentration. (Appx2469-2471.)

G. The Trial

At trial, after this supplemental claim construction, Apotex again changed its position on the total protein concentration of its refold mixture. After Amgen's technical expert completed his testimony about the protein concentration of Apotex's refold mixture, Apotex presented yet another new theory of non-infringement. According to Apotex employee (and fact witness) Dr. Jason Dowd, the 0.9-1.4 g/L inclusion-body concentration of its refold mixture does not represent the total protein concentration because Apotex's inclusion bodies allegedly are approximately two-thirds water (Apotex's "water theory").[1] (Appx3610-3611, Appx3644-3645.) This results in a <u>total</u> protein concentration (in contrast to the filgrastim protein concentration about which Dr. Robinson had testified) of about 0.3-0.5 g/L in the refold mixture. The district court recognized

[1] The district court's Findings of Fact and Conclusions of Law incorrectly describe Dr. Dowd as Apotex's expert. (*See* Appx23-24.)

that this position was new: "this opinion apparently ha[d] not been disclosed by the defendant prior to this time." (Appx3467-3473.) This inclusion-body water content was not a reflection of expert opinion but rather was derived by Dr. Dowd from batch records generated by Intas, Apotex's contract manufacturer. (*See* Appx3643-3645 (discussing Appx4250-4251).) At no point did Dr. Dowd testify he had actually measured the water content of Intas's inclusion bodies. (*See* Appx3611.) Apotex also presented the new theory, through its employee, Dr. Dowd, that, if measured by chromatography, the maximum total protein concentration in its refold mixture would be 0.708 g/L. (Appx3620-3621; Appx4790.)

H. What Apotex's aBLA Specifications Say About Protein Concentration

Apotex's aBLAs provide that Apotex's inclusion bodies, before being added to the refold mixture, are successively washed three times to remove non-protein contaminants and, after each wash, centrifuged at over 10,000 times the force of gravity for thirty minutes to separate a "pellet" of inclusion bodies from the supernatant (liquid). (*E.g.*, Appx5582-5587; Appx5892-5897.) The concentration of these washed and centrifuged inclusion bodies is referred to as the "washed-inclusion-body concentration" throughout this brief. Apotex's aBLA specifications disclose that the washed-inclusion-body concentration of Apotex's refold mixture is 0.9-1.4 g/L. (Appx5592-5594; Appx5900-5902.) The aBLA

specifications are silent about the water content of Apotex's inclusion bodies. Indeed, the 0.9-1.4 g/L inclusion-body concentration, repeated at least four times, is never designated as a "wet weight." (Appx5592-5594; Appx5900-5902; Appx6728; Appx7359.) In contrast, when discussing the prior step of Apotex's manufacturing process which results in the washed inclusion bodies, the aBLAs describe one of the performance parameters as an "inclusion body wet weight" with an expected range of 2.8-4.0 g/L. (Appx5588; Appx5896.)

Further, the 0.9-1.4 g/L inclusion-body concentration of Apotex's refold mixture, as well as the 2.8-4.0 g/L "inclusion body wet weight" concentration of the prior step, are designated as "Key Process Parameters," or "KPPs," which by definition may be exceeded. (Appx5588; Appx5896; Appx6728; Appx7359.) The aBLAs provide:

> **A Key Process Parameter (KPP)** is process parameter that, if varied within the characterization range, results in significant variation in process consistency, but less significant variation in a Product Quality Attribute. These parameters should be carefully controlled within a narrow range and are essential for process performance. <u>If the acceptable range is exceeded</u>, it may affect the process (e.g., yield, duration), but not product quality.

(Appx6724-6725 (emphasis added); Appx7353(emphasis added).) This stands in contrast to Critical Process Parameters, or "CPPs," with which strict compliance is required. (Appx6724-6725; Appx7353.) KPPs do not receive as much weight as CPPs, as illustrated by the fact that each CPP has an associated "Acceptance

Criterion," whereas the KPP upon which Apotex relies for its assertions about protein concentration in its refold mixture merely has an "Expected Range." (Appx5595; Appx5902.)

Additionally, the aBLA specifications state the "Expected Range" for the filgrastim concentration in Apotex's protein-containing "volume," i.e, before it becomes part of Apotex's refold mixture: 4.24-11.80 mg/ml. (*See* Appx5595; Appx5902.) This range is measured using chromatography techniques. (*See* Appx5595; Appx5902.) The aBLAs state that the "Solubilized IB [Inclusion Body] purity" by chromatography is greater than or equal to 75%. (Appx5595; Appx5902.) These are the values that Dr. Dowd used during his trial testimony to calculate the 0.708 g/L value that he called the maximum total protein concentration of Apotex's refold mixture. (Appx3617-3621; Appx4790.) The aBLAs designate the 4.24-11.80 mg/ml range as a KPP, which, as discussed, may be exceeded. Indeed, when denying Apotex's summary-judgment motion, the district court expressly acknowledged that the 4.24-11.80 mg/ml KPP can be exceeded. (Appx2471.)

I. The District Court's Decision

On September 6, 2016, the district court issued its findings of fact and conclusions of law, and its final judgment. The court entered judgment in favor of Apotex and against Amgen on Amgen's claims of infringement and on Apotex's

counterclaims regarding non-infringement. (Appx43-47.) The court found that Apotex's refolding process does not include a refold mixture having a protein concentration at or above about 1 g/L. (Appx22.) The court adopted Apotex's "water theory," finding that "Apotex's inclusion bodies are composed of approximately two-thirds water at the time of weighing." (Appx24.) The court also found that Apotex's refolding process does not include a redox component having a redox buffer strength of 2 to 100 mM or its equivalent. (Appx28.) The court assumed, without deciding, that Apotex's process meets all of the limitations of this element, except that "Amgen has not proven by a preponderance of the evidence that the redox buffer strength of Apotex's hypothetical redox component [i.e., 214 mM to 340 mM] is insubstantially different from the claimed redox buffer strength of 2 to 100 mM." (Appx29-30.)

SUMMARY OF THE ARGUMENT

The district court erred in finding that Apotex's equivalent redox component does not satisfy the claimed redox-buffer-strength limitation.

First, the district court erroneously construed the term "2 mM or greater" of claim 1 of the '138 Patent to mean "2 mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100 mM." (Appx9-10.) The court imported a limitation into the claims based on disclosure that pertains only to specific embodiments of the invention. Furthermore, neither the specification nor prosecution history otherwise provides a basis for imposing an effective upper limit of 100 mM on redox buffer strength. The plain wording of the claim term and the specification support "2 mM or greater" as meaning exactly that: 2 mM or greater, without an upper bound. Moreover, as the district court found (Appx28-29), the specification discusses "effective" maximum boundaries because of the "solubility limitations" of certain solutes in certain solvents: there is a maximum amount of a redox chemical that can be dissolved in a particular solvent. But, this "effective" maximum boundary varies depending on choice of redox chemical and choice of solvent. There is no reason to depart from the plain meaning of the claim term "2 mM or greater" merely because the specification points out that for certain embodiments, the "effective" maximum redox buffer strength is 100 mM. Under the term's proper construction, the redox buffer strength of Apotex's redox

component falls within the claim limitation because Apotex's redox buffer strength is 214-340 mM, i.e., greater than 2 mM. (Appx30.)

Second, even if this Court agrees with the district court's construction of "2 mM or greater" as having an upper boundary of 100 mM, the district court's finding of fact that Apotex's process does not equivalently meet this limitation is clearly erroneous. Amgen offered ample evidence at trial that Apotex infringes the "2 mM or greater" limitation equivalently, including showing that Apotex's equivalent 472 mL redox component with a redox buffer strength ranging from 214-340 mM delivers the same amount of oxidants and reductants to the 160 L refold mixture as would a 1-1.6 L redox component with a redox buffer strength of 100 mM (within the scope of claim 1). Apotex did not counter this evidence. Instead, Apotex's rebuttal evidence focused on the existence of differences between Apotex's redox component and a redox component having a redox buffer strength of 100 mM, in other words, the fact that Apotex does not infringe this limitation literally. The self-evident fact that there is no literal infringement cannot by itself amount to a showing of no infringement under the doctrine of equivalents. By relying on this evidence, the district court committed legal error, improperly turning the equivalents analysis into one of literal infringement.

For these reasons, the district court erred in finding noninfringement of the "2 mM or greater" limitation. Amgen respectfully requests that this Court reverse

the district court's judgment of non-infringement, or, at a minimum, reverse the district court's claim-construction order, and remand for further proceedings.

* * * *

The district court also erred in finding that Apotex's refolding process does not include a refold mixture having a protein concentration at or above about 1 g/L.

First, the district court erroneously declined to give any weight whatsoever to the facts asserted by Apotex in its subparagraph 262(*l*)(3)(B) statements. (*See* Appx24, Appx34-35.) The BPCIA requires the Applicant to provide a "detailed statement" of the "factual and legal basis" for its assertion that the patent in question will not be infringed. 42 U.S.C. § 262(*l*)(3)(B). The patent holder then relies upon this detailed statement in drafting its subparagraph 262(*l*)(3)(C) statement and in deciding whether and on which patents to bring suit. If the detailed statement required by the BPCIA can simply be repudiated without explanation, as the district court permitted Apotex to do here, the entire purpose of the pre-litigation exchange is subverted. In its detailed statement, Apotex unequivocally represented that its refold mixture contained 0.9-1.4 g/L of the protein of interest, filgrastim—a concentration that falls within the limitation of claim 1 of the '138 Patent, "at or above about 1 g/L." It was error of law for the district court to accord no weight to this statement in making its decision. Moreover, this is an issue that if not corrected now, is likely to recur in subsequent

infringement actions under the BPCIA. Accordingly this court should hold that facts asserted in a detailed statement under subparagraph 262(*l*)(3)(B) are entitled to weight as a matter of law, and remand for further fact finding.

Second, the district court also erred in finding that the "about 1 g/L" floor protein concentration in the refold mixture, as required by claim 1 of the '138 Patent, is not interchangeable with washed-inclusion-body concentration. The language "about 1 g/L" does not appear in the claims and was proposed by Amgen in response to Apotex's effort to read in a minimum protein concentration of 2 g/L in the refold mixture. The requirement of "about 1 g/L" is necessarily based on the disclosure of the '138 Patent specification and that disclosure makes clear the patent considers protein and washed inclusion bodies to be interchangeable. Indeed, before trial, Apotex had never disputed their interchangeability. Rather, in accordance with the specification of the '138 Patent, both parties had viewed the 0.9-1.4 g/L inclusion-body concentration in Apotex's refold mixture as a measure of total protein concentration in the refold mixture. Only at trial did Apotex unveil its theory that its inclusion bodies are composed of approximately two-thirds water. By withholding this theory until trial, Apotex deprived Amgen of the ability to argue during the claim-construction phase of this case that washed-inclusion-body concentration is interchangeable with protein concentration. Accordingly, Amgen respectfully requests that this Court reverse

the district court's de facto determination that the "about 1 g/L" floor protein concentration in the refold mixture is not interchangeable with the washed-inclusion-body concentration, and that this Court remand for further proceedings.

Third, Apotex's aBLA specifications permit the refold mixture in Apotex's manufacturing process to have a protein concentration "at or above about 1 g/L protein," in accordance with the district court's claim construction. (Appx9.) In the Hatch-Waxman context the law is clear that "if a product that an ANDA applicant is asking the FDA to approve for sale falls within the scope of an issued patent, a judgment of infringement must necessarily ensue." *Sunovion Pharm., Inc. v. Teva Pharm. USA, Inc.*, 731 F.3d 1271, 1278 (Fed. Cir. 2013). The same principle should apply under the BPCIA: if the aBLA specifications permit a product or process in question to fall within the scope of a patent claim, that should be an infringement as a matter of law. By finding that the maximum protein concentration in Apotex's refold mixture was "limited" to 0.708 g/L, and by considering evidence extrinsic to the aBLA specifications themselves, including batch records and fact-witness testimony regarding the batch records, the district court erred in concluding there is no infringement.

For these reasons, the district court erred in its determination of noninfringement of the limitation that the refold mixture have a protein

concentration "at or above about 1 g/L." Amgen respectfully requests that this Court reverse the district court's judgment and remand for further proceedings.

ARGUMENT

I. The District Court Erred in Narrowly Construing the Redox-Buffer-Strength Limitation and Then Finding It Was Not Satisfied by Apotex's Equivalent Redox Component

The district court erroneously construed the term "2 mM or greater" to include an effective maximum boundary of 100 mM. For this reason alone, it erred in finding that Apotex's equivalent redox component fails to satisfy this redox-buffer-strength limitation of claim 1. This Court should reverse. The refold buffer strength of Apotex's equivalent redox component is 214-340 mM (Appx30)—i.e., "2 mM or greater." (Appx65.)

Alternatively, even if "2 mM or greater" is construed to have an effective maximum boundary of 100 mM, the district court clearly erred in finding that Apotex's manufacturing process does not meet the redox-buffer-strength limitation of claim 1 under the doctrine of equivalents.

A. The District Court Erred in Construing the Claim Term "2 mM or Greater"

Claim 1 of the '138 Patent includes the following limitation: a "redox buffer strength of 2 mM or greater." (Appx65.) The district court construed "2 mM or greater" to mean "2 mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100 mM," reading in a limitation found nowhere in the claim. (Appx9-10.) This Court reviews claim-construction rulings de novo when based solely on the intrinsic record. *See Teva Pharm. USA, Inc. v. Sandoz, Inc.*,

135 S. Ct. 831, 841 (2015). In this case, the intrinsic record fully determines the proper construction. Therefore this Court should review the district court's construction de novo.

1. "2 mM or Greater" Plainly Means 2 mM or Greater, with No Maximum Boundary

"[W]ords of a claim are generally given their ordinary and customary meaning" which is "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (internal quotations omitted). "2 mM or greater" should be given its ordinary meaning: 2 mM or greater. "When claim language has as plain a meaning on an issue as the language does here, leaving no genuine uncertainties on interpretive questions relevant to the case, it is particularly difficult to conclude that the specification reasonably supports a different meaning." *Straight Path IP Grp., Inc. v. Sipnet EU S.R.O.*, 806 F.3d 1356, 1361 (Fed. Cir. 2015). Further, the "specification plays a more limited role than in the common situation where claim terms are uncertain in meaning in relevant respects." *Id.* Here, "2 mM or greater" is not uncertain in meaning, and "stay[ing] true to the claim language," *Phillips*, 415 F.3d at 1316, the term "2 mM or greater" means 2 mM or greater.

The specification introduces concept of a maximum boundary in the context of an embodiment, not as a limitation on the invention. Indeed, the patent's

Summary of the Invention section first describes the invention as a method comprising a redox buffer strength of 2 mM or greater, without mention of an effective maximum boundary. (Appx57.) The next paragraph discusses "various embodiments" and introduces exemplary ranges of redox buffer strengths and the effective maximum boundary of 100 mM. (Appx57-58.) Accordingly, the patent never describes the effective maximum boundary "as the present invention, as essential, or as important." *See GE Lighting Sols., LLC v. AgiLight, Inc.*, 750 F.3d 1304, 1309-10 (Fed. Cir. 2014); *see also Medegen MMS, Inc. v. ICU Med., Inc.*, 317 F. App'x 982, 986 (Fed. Cir. 2008) (nonprecedential) (looking to the summary of the invention section to determine whether to read a limitation into a claim term).

Moreover, the patentees knew how to include language limiting the redox buffer strength. The specification, in the context of particular embodiments, several times repeats "a Thiol-pair buffer strength [i.e., redox buffer strength] equal to or greater than 2 mM, <u>for example</u> greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM." (*E.g.*, Appx57-58 (emphasis added).) And these lower boundaries, from 2.25 mM to 15 mM, are recited in claim 3. (Appx65.) But, neither claim 1 nor claim 3 includes

the upper boundary of 100 mM. The only reasonable conclusion is that no such boundary is intended.

The district court explained why its construction appended the additional phrase "wherein the redox buffer strength is effectively bounded at a maximum of 100 mM," stating that the "specification repeatedly sets forth a suggested range of redox buffer strengths, yet each time specifically limits the possible ranges" to an effective maximum boundary of 100 mM. (Appx10.) Reading in such limitations is improper, even when, as here, the specification discloses several embodiments that include the limitation that is, nonetheless, absent from the claims. *See Epos Techs. Ltd. v. Pegasus Techs. Ltd.*, 766 F.3d 1338, 1343-44 (Fed. Cir. 2014) (rejecting claim constructions that improperly included limitations repeatedly disclosed in the specification, where the specification did not clearly indicate that the claims should include such limitations).

This is unlike *SciMed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc.*, 242 F.3d 1337 (Fed. Cir. 2001), on which the district court relied. (Appx10.) In that case, the abstract, summary of the invention, and conclusion sections of the specifications describe the invention as containing the limitation at issue; the specifications distinguish the prior art because it lacked the limitation; and the specifications stated that the limitation was included in "all embodiments of the present invention contemplated and disclosed herein." *SciMed*, 242 F.3d at

1342-43. In contrast, no such language is present in the specification of the '138 Patent.

2. The District Court, After Trial, Found that the "Effective Maximum Boundary" Mentioned in the Patent Specification Addresses Solubility Limitations, Which Should Not Limit the Plain Meaning of the Claim Language

The patent specification does not explain why the 100 mM redox buffer strength is an "effective" boundary. However, the district court found that the "imposition of an effective maximum redox buffer strength is to address solubility limitations." (Appx29 (emphasis added).) In other words, depending on the solvent used to dissolve the redox-component chemicals, the redox buffer strength may have an "effective" maximum boundary simply because there is a maximum amount of any given chemical that can be dissolved in a particular solvent. (*See* Appx9613-9614; *see* Appx3513-3514.) This "effective" maximum boundary, however, varies depending on the materials being dissolved and choice of solvent. For instance, here, Apotex dissolves 13.2 ± 3.6 g of cystine in 440 mL of 0.2 N hydrochloric acid (Appx5600; Appx5906), resulting in a concentration of cystine ranging from 85 mM to 148 mM. (Appx3146.) Therefore, the effective maximum concentration of cystine in 0.2 N hydrochloric acid is greater than 100 mM. The plain meaning of the claim term "2 mM or greater" should not be limited merely because the patent specification points out that, for certain embodiments, the effective maximum redox buffer strength is 100 mM.

In contrast, the minimum redox buffer strength—i.e., 2 mM—is a required claim limitation. First, the minimum redox buffer strength is expressly called for in the claim language. (Appx65.) Second, the patent specification states (and the district court found) that "[a]t lower redox buffer strengths, the overall system becomes much more difficult to control." (Appx28-29; Appx60.) The specification does not make a similar disclosure with respect to redox buffer strengths greater than 100 mM, and accordingly, such an effective maximum boundary should not be read into the claim.

The specification also explains that the "[o]ptimization of the buffer thiol strength [i.e., the redox buffer strength] . . . can be tailored to a particular protein." (Appx58, Appx61.) This indicates to a skilled artisan that, depending on the protein to be refolded, the optimal redox buffer strength can be determined empirically, without a strict upper bound of 100 mM. The examples in the specification also repeatedly indicate that, during the initial testing to identify the optimal redox buffer strength, a sufficiently large range of redox buffer strengths may be tested. (*See, e.g.*, Appx61-62.) The "2mM or greater" claim language accommodates the possibility that the refold buffer strength may exceed the 100 mM value associated with specific embodiments.

B. Even if the Redox Buffer Strength Is Effectively Bounded at a Maximum of 100 mM, the District Court Clearly Erred in Holding that Apotex's Redox Component Does Not Satisfy This Limitation Equivalently

Even if this Court were to accept the district court's construction of "2 mM or greater," the district court's finding that Apotex's process does not equivalently meet this limitation is clearly erroneous.

Following claim construction, Amgen asserted infringement under the doctrine of equivalents. "What constitutes equivalency must be determined against the context of the patent, the prior art, and the particular circumstances of the case." *Graver Tank & Mfg. Co. v. Linde Air Prods. Co.*, 339 U.S. 605, 609 (1950); *accord Warner-Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 40 (1997).

The test for equivalence has been framed in two ways. The "insubstantial differences" test examines whether an asserted equivalent "plays a role substantially different from the claimed element." *Warner-Jenkinson*, 520 U.S. at 40. The "function-way-result" test examines "whether a substitute element matches the function, way, and result of the claimed element." *Id.* Which test to apply depends on the facts of the case, because "[d]ifferent linguistic frameworks may be more suitable to different cases." *Id.*

The patentee must demonstrate infringement under the doctrine of equivalents by a preponderance of the evidence. *Siemens Med. Sols. USA, Inc. v. Saint-Gobain Ceramics & Plastics, Inc.*, 637 F.3d 1269, 1279 (Fed. Cir. 2011).

This Court reviews infringement determinations for clear error following a bench trial. *Golden Blount, Inc. v. Robert H. Peterson Co.*, 365 F.3d 1054, 1058 (Fed. Cir. 2004). "A factual finding is clearly erroneous if, despite some supporting evidence, [the Court is] left with the definite and firm conviction that a mistake has been made." *Ferring B.V. v. Watson Labs., Inc.-Fla.*, 764 F.3d 1401, 1406 (Fed. Cir. 2014).

Amgen offered ample evidence at trial that Apotex infringes the "2 mM or greater" limitation equivalently. Apotex did not counter this evidence. Instead, Apotex's rebuttal evidence focused on the literal differences between Apotex's redox component and a redox component having a redox buffer strength effectively bounded at a maximum of 100 mM, and provided no explanation for why these differences have any significance other than the *ipse dixit* that they are substantial. (*See* Appx3458-3461.) Therefore, the district court clearly erred in adopting Apotex's flawed literal-infringement-type analysis and finding that Amgen had not met its burden to prove that Apotex infringes the redox-buffer-strength limitation equivalently (*see* Appx28-31, Appx36-38). Further, the court erred as a matter of law by misapplying the doctrine of equivalents so that the only way there could be infringement was literally (*see* Appx38). *See Deere & Co. v. Bush Hog, LLC*, 703 F.3d 1349, 1356-57 (Fed. Cir. 2012).

1. Amgen's Evidence Shows that Apotex's Process Satisfies the Redox-Buffer-Strength Limitation Equivalently

The redox buffer strength of claim 1, as construed by the district court, is effectively bounded at a maximum of 100 mM. (Appx10.) At trial, Amgen offered ample evidence that Apotex's 472 mL redox component[2] with a redox buffer strength of 214-340 mM is insubstantially different from a redox component within the scope of the claim as construed by the court, namely a 1.0-1.6 L redox component having a redox buffer strength of 100 mM. As Amgen's expert Dr. Willson testified, there is no "practical consequence" to using a smaller volume of a more concentrated redox component. (*See* Appx3147.) Similarly, the inventor Dr. Hart testified that if the redox buffer strength exceeds 100 mM, "nothing adverse would happen" if all components stayed in solution. (Appx3013.) This is so because the goal in selecting a redox buffer strength is "to deliver a selected mass of some dissolved material, the dissolved cysteine and dissolved cysteine [sic, cystine]" to the refold mixture. (Appx3149.) Claim 1 is not limited with respect to either the volume of the redox component or the volume

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[2] Apotex introduces oxidant and reductant into its refold mixture by adding its Cystine Solution and Cysteine Solution in a stepwise manner rather than first premixing the solutions into a single redox-component and then adding the premixed solution to the refold buffer. (Appx3124-3125; Appx5597-5599; Appx5904-5905.) The combined volume of these two solutions is approximately 472 mL. (Appx29.) For purposes of its opinion, the district court "assume[d], without deciding that the Cysteine and Cystine Solutions added in a stepwise manner in Apotex's refolding process is the equivalent of the claimed redox component." (*Id.*)

of the refold mixture. (Appx65.) Apotex's 472 mL redox component with a redox buffer strength of 214-340 mM would deliver the same mass of dissolved cysteine and cystine to its 160 L refold mixture as would a 1.0-1.6 L redox component with a redox buffer strength of 100 mM (within the redox-buffer-strength limitation of the claim). (Appx3150, Appx3154; *see* Appx9974-9975.) Further, to achieve proper refolding, Apotex's redox component is ultimately added to the refold mixture, which has a much larger volume of 160 L. (*E.g.*, Appx5597-5599; Appx5904-5905.) Whether the volume of the redox component is 472 mL (Apotex's process) or 1.0-1.6 L (within the literal scope of the claimed process), it makes up less than 1% of a 160 L refold mixture, indicating that the difference in volume between Apotex's 472 mL refold mixture and a 1.0-1.6 L refold mixture is insubstantial. (Appx3154; *see* Appx9976.)

Likewise, Dr. Willson explained that Apotex's redox component with a redox buffer strength of 214-340 mM performs the same function in substantially the same way to achieve the same result as a redox component having a redox buffer strength effectively bounded at a maximum of 100 mM. Specifically, the <u>function</u> of the redox buffer strength of the redox component is a measure of oxidants and reductants that will ultimately end up in the refold mixture. (*See* Appx3149.) Here, both redox components deliver the same numbers of molecules of cystine and cysteine to the refold mixture. (Appx3150.) Whether 472 mL

(Apotex's process) or 1.0-1.6 L (claimed process) of redox component is added to a 160 L refold mixture, it is done in substantially the same way: the total volume of the refold mixture is not substantially altered. (Appx3154.) Finally, the addition of a 472 mL redox component with a 214 mM to 340 mM redox buffer strength to a 160 L refold mixture achieves the same result as the addition of a 1.0 L to 1.6 L redox component with a 100 mM redox buffer strength to a 160 L refold mixture. The number of added oxidant and reductant molecules is the same, and the slight variation in total volume of the refold mixture would not significantly, if at all, impact protein refolding. (Appx3151-3152.)

2. The District Court Erred by Instead Crediting Apotex's Testimony, Which Is Factually Insufficient and Irrelevant to the Doctrine-of-Equivalents Analysis

The district court clearly erred in "credit[ing] Dr. Robinson's opinion" and finding that the difference between Apotex's process and the claimed process is substantial (*see* Appx30). Dr. Robinson's trial testimony and the district court's opinion focus on the differences between Apotex's redox component and a redox component with a redox buffer strength effectively bounded at a maximum of 100 mM—namely (1) that the 214-340 mM redox buffer strength of Apotex's redox component is two to three times higher than the claim's effective maximum of 100 mM, as construed by the court, and (2) that the 472 L volume of Apotex's redox component is approximately 0.5 to 1 L smaller than the volume of 1.0-1.6 L

that would be required for a comparable redox component diluted to a redox buffer strength of 100 mM. (Appx30; Appx3458-3459.) However, these are just mathematical differences. Pointing out the differences between Apotex's redox component and a redox component that literally satisfies the redox-buffer-strength limitation, without more, simply supports a finding of no literal infringement. Dr. Robinson provided no testimony as to why these differences are <u>substantial</u>. In fact, at her deposition Dr. Robinson was unable to point to any technical phenomenon that would differ between a redox component with a redox buffer strength below 100 mM and one with a redox buffer strength exceeding 100 mM. (Appx9615; *see* Appx3516-3517.)

The district court noted in its opinion that Dr. Willson did not conduct any experiments to show that a 1.0-1.6 L redox component with a 100 mM redox buffer strength would work with Apotex's process. (Appx30-31; *see* Appx3457.) This criticism misapprehends the equivalents inquiry. There is no requirement that experiments be conducted in an equivalents analysis. Further, the issue was never whether a redox component with a volume of 1.0-1.6 L and a redox buffer of 100 mM would work in Apotex's process. Rather, the equivalents inquiry was whether Apotex's use of a smaller volume of more concentrated material makes its process substantially different from the process claimed in claim 1 of the '138 Patent. It does not.

That "Dr. Willson did not specify what liquid would be used to increase the volume of the hypothetical redox component in Apotex's process to achieve the desired redox buffer strength" (Appx30; *see* Appx3459) is also inapposite. Claim 1 of the '138 Patent is not limited with respect to the identity of the solvent used in the redox component. (Appx65.)

Likewise, the district court's finding that "Dr. Willson also acknowledged that he did not know where equivalence would be lost by increasing the volume of the redox component" (Appx30; *see* Appx3460-3461) is not relevant to the specific question of whether Apotex's process is equivalent to the claimed process. No precedent of this Court requires evidence specifying a precise boundary at which equivalence is lost.

Further, in holding that Apotex does not infringe the redox-buffer-strength limitation equivalently, the district court stated that "adjusting the volume of the hypothetical redox component to reach a desired redox buffer strength <u>that is not actually utilized in Apotex's process</u> renders meaningless the maximum limit of 100 mM because one could simply adjust the volume of any redox component with a redox [buffer strength] greater than 100 mM to make it fall within the claimed limitation." (Appx38 (emphasis added).) This statement erroneously suggests that the only way one could infringe the redox-buffer-strength limitation would be by <u>actually utilizing</u> a redox component with a redox buffer strength between 2 mM

and 100 mM—in other words, literal infringement. This is wrong as a matter of law. "[T]he vitiation test cannot be satisfied by simply noting that an element is missing from the claimed structure or process because the doctrine of equivalents, by definition, recognizes that an element is missing that must be supplied by the equivalent substitute." *Deere & Co.*, 703 F.3d at 1356-57; *accord Brilliant Instruments, Inc. v. GuideTech, LLC*, 707 F.3d 1342, 1347 (Fed. Cir. 2013).

Lastly, the district court commented that "Apotex is bound by the specifications in its aBLAs and cannot, in practice, increase the volume of its redox component to a volume of 1.0 to 1.6 L without facing serious legal penalties." (Appx38; *see* Appx31.) This again misapprehends the nature of the equivalents analysis. Regulatory law is irrelevant here. The inquiry was not whether, as a matter of FDA law, Apotex's process could be changed to something within the scope of the claim, but rather, as a matter of patent law, was Apotex's process the equivalent of something that is within the scope of the claim.

II. The District Court Erred in Finding that Apotex's Refolding Process Does Not Include a Refold Mixture Having a High Protein Concentration, at or Above About 1 g/L

The district court erred in finding that Apotex's refold mixture does not have a high protein concentration, "at or above about 1 g/L," for three independent reasons.

A. Apotex's Admissions in Its Statements Pursuant to Subparagraph 262(*l*)(3)(B) of the BPCIA Are Highly Probative of Apotex's Infringement of the '138 Patent

Pursuant to the BPCIA's required information exchanges, Apotex provided Amgen with "detailed statement[s] of the factual and legal bases that, in its opinion and to the best of its knowledge," the claims of the '138 Patent "will not be infringed by the commercial marketing" of Apotex's pegfilgrastim and filgrastim products. (Appx7382; Appx7427.) *See* 42 U.S.C. § 262(*l*)(3)(B). In these exchanges, Apotex twice represented to Amgen (first on April 17, 2015 for its pegfilgrastim product and later on June 29, 2015 for its filgrastim product) that "As described in Apotex's manufacturing documents, the concentration of its [filgrastim] in the refold buffer is 0.9-1.4 g/L." (Appx7396; Appx7447.) The "concentration of its [filgrastim] in the refold buffer" in Apotex's process is the same as the concentration of filgrastim in the refold mixture of claim 1 because Apotex adds the protein-containing "volume" to its "Refolding Buffer" to form the "refold mixture." (*See* Appx5597; Appx5904.) Apotex's filgrastim concentration in its refold mixture (0.9-1.4 g/L), therefore, falls within the protein-concentration limitation of the "refold mixture" of claim 1, as construed by the district court: i.e., "The refold mixture has a high protein concentration, where 'high protein concentration' is at or above about 1 g/L protein." (Appx9.)

Amgen should be permitted to rely on Apotex's factual admissions made during the BPCIA exchanges to prove infringement, just as it relied on those admissions in bringing the action in the first instance, throughout fact and expert discovery, when it opposed Apotex's summary-judgment motion, and when it presented its case in chief at trial. Only an Apotex employee, not an expert, Dr. Dowd, who had no role in preparing the statements, disputed the representations in Apotex's subparagraph 262(*l*)(3)(B) statements, during Apotex's rebuttal case at trial. (*See* Appx3660-3668.) What is more, Dr. Dowd relied on reports generated by Apotex's contract manufacturer, not his personal knowledge, and testified that he had no personal knowledge as to the consistency of Apotex's inclusion bodies. (*See* Appx3624-3627, Appx3660-3665.) Indeed, he never inspected the inclusion bodies; he only observed them "through a window into the operational area" at Apotex's contract manufacturer in India. (Appx3664-3665.) Other than this testimony at trial, Apotex never sought to retract its subparagraph 262(*l*)(3)(B) representations or to explain why those representations were incorrect. The district court, however, refused to consider the factual representations "probative on the issue of protein concentration," effectively making the BPCIA exchange process irrelevant in a subsequent litigation. (Appx24, Appx34-35.) This was legal error.

1. Statements Pursuant to Subparagraph 262(*l*)(3)(B) of the BPCIA Must Be Accurate; Otherwise, the BPCIA Information-Exchange Process Is a Meaningless Exercise

The BPCIA "established a unique and elaborate process for information exchange between the biosimilar Applicant and the [Sponsor] to resolve patent disputes." *Id.* at 1352. That process is embodied in 42 U.S.C. § 262(*l*), "Patents." Within 20 days after FDA notifies a biosimilar Applicant that its aBLA has been accepted for review, the Applicant may give the Sponsor a copy of its aBLA and other manufacturing information. 42 U.S.C. § 262(*l*)(2)(A); *Sandoz*, 794 F.3d at 1352.

Next follows a sequential exchange of "lists of patents for which" the parties "believe a claim of patent infringement could reasonably be asserted by the [Sponsor]." *Sandoz*, 794 F.3d at 1352. The Sponsor initiates the exchange with a patent list in accordance with subparagraph 262(*l*)(3)(A). The Applicant "may" respond with its own list of additional patents that could be infringed. 42 U.S.C. § 262(*l*)(3)(B). However, it "shall provide" for each listed patent either a statement that it will remain off the market until the patent expires or, on a claim-by-claim basis, a "detailed statement" of its "factual and legal basis" for believing that the patent is invalid, unenforceable, or not infringed. *Id.* Finally, the Sponsor then "shall provide" for the disputed patents a detailed statement that each patent will be

infringed and a response to the Applicant's invalidity and unenforceability contentions. *Id.* § 262(*l*)(3)(C).

The next step is for the parties—informed by the prior exchange—to attempt to agree, under paragraph 262(*l*)(4), on which of the patents listed pursuant to paragraph 262(*l*)(3), if any, should be included in an immediate patent-infringement action and, failing agreement, to follow a procedure under paragraph 262(*l*)(5) to identify those patents. Either way, once the parties have this list of patents, the Sponsor is then directed to bring an "[i]mmediate patent infringement action" on each listed patent within 30 days. *Id.* § 262(*l*)(6); *Sandoz*, 794 F.3d at 1352.

The information exchange in paragraphs 262(*l*)(2) and (3) is fundamental to the BPCIA purpose of efficient resolution of patent issues. *See Apotex*, 827 F.3d at 1063; *see also Sandoz*, 794 F.3d at 1364 (Newman, J., dissenting in part). Indeed, the Sponsor is expected to and does rely on the accuracy of the information it receives from the Applicant in this exchange. It is key to which patents are litigated and when. Accordingly, the consequences to the Sponsor of a subsequent retraction of a stated fact in the Applicant's subparagraph 262(*l*)(3)(B) statement—especially a retraction during trial—are severe.

2. Amgen Should Be Permitted To Rely on Apotex's Admissions in its Subparagraph 262(*l*)(3)(B) Statements To Prove Infringement

Here, Amgen proceeded with litigation in reliance on the truth of Apotex's representation that the concentration of the protein of interest in the refold mixture was 0.9-1.4 g/L. For example, Amgen expressly called out this representation in its responsive detailed statement pursuant to subparagraph 262(*l*)(3)(C) explaining why there was infringement. (Appx11225; Appx11269.) Thereafter Amgen expended millions of dollars litigating, up to and including a trial. It cannot be the case that a biosimilar applicant can simply disavow its own statements made during the statutory exchange, especially after such detrimental reliance on the part of the Reference Product Sponsor. Material factual representations by an Applicant in its subparagraph 262(*l*)(3)(B) statements should be treated as admissions, and not casually brushed aside as non-binding and non-probative, as the district court did. Otherwise, the purposes of the statutory information exchange—efficient resolution of patent issues—would be thwarted.

This Court reviews the statutory interpretation of the BPCIA de novo. *See Sandoz*, 794 F.3d at 1354. However, the issue of whether and to what extent probative value should be accorded to factual information disclosed by a biosimilar Applicant in its subparagraph 262(*l*)(3)(B) statement has not previously been addressed by this Court. Since the issue is one of law, and indeed implicates the

meaning and purpose of the BPCIA itself, Amgen respectfully suggests the appropriate standard is one of de novo review.

The district court erroneously called Apotex's subparagraph 262(*l*)(3)(B) statements "not probative on the issue of protein concentration," "not binding on Apotex," and "not controlling": "These letters were not part of Apotex's aBLAs, were never filed with the FDA, do not impact the process and product approved by the FDA, and are not controlling." (Appx24, Appx34.) Accordingly, the district court erroneously failed to accord any probative value to the information disclosed during the BPCIA exchanges.

The district court cited *Takeda Chem. Indus. Ltd. v. Mylan Labs. Inc.*, 549 F.3d 1381, 1390-91 (Fed. Cir. 2008) for the proposition that generic drug applicants under the Hatch-Waxman Act are not necessarily limited to the theories raised in their paragraph IV certification letters. (Appx34-35.) Under the Hatch-Waxman Act, applicants seeking approval to market a generic version of a small-molecule drug may submit, with their application, a paragraph IV certification that includes "a detailed statement of the factual and legal basis of the opinion of the applicant that the patent is invalid or will not be infringed." 21 U.S.C. § 355(j)(2)(B)(iv)(II). Even assuming that the notice in a paragraph IV letter is directly analogous to a statement under subparagraph 262(*l*)(3)(B) of the BPCIA, *Takeda* is inapposite. Apotex did not assert additional <u>legal theories</u> absent from

its subparagraph 262(*l*)(3)(B) statements. Rather, Apotex sought to change the facts as presented in its subparagraph 262(*l*)(3)(B) statements regarding the manufacturing process described in its aBLAs: specifically, that the filgrastim concentration in its refold mixture ranged from 0.9-1.4 g/L. It is one thing to introduce new legal positions as a case develops. It is another thing entirely to retract the facts that formed the basis of the lawsuit in the first instance.

Nowhere does *Takeda* (or any other authority) say that an applicant is permitted to change the facts in its paragraph IV certification. In fact, under the Hatch-Waxman Act, FDA has the authority to reject an application if the information in the paragraph IV certification is inaccurate. *See* 21 U.S.C. § 355(j)(2)(A)(vii)(IV), (4)(K). FDA need not approve an application if "the application contains an untrue statement of material fact." *Id.* § 355(j)(4)(K). The accuracy of paragraph IV certifications, therefore, as part of the "application," may be ensured by FDA. *See id.* § 355(j)(2)(A)(vii)(IV); *see also Dr. Reddy's Labs., Inc. v. Thompson*, 302 F. Supp. 2d 340, 352 (D.N.J. 2003).

Apotex twice reported that the filgrastim concentration in its refold mixture was 0.9-1.4 g/L—once in its pegfilgrastim statement and again in its filgrastim statement. (Appx7396; Appx7447.) Each time, Apotex cited its own aBLAs, specifically, the portions stating that the solubilized inclusion bodies are "diluted to

160L with refolding buffer," which "results in a final IB concentration of 0.9-1.4 g/L of refolding buffer." (*See* Appx5593; Appx5901.)

When Apotex made its subparagraph 262(*l*)(3)(B) statements, the district court had not yet construed the '138 Patent claims to require a high protein concentration at or above about 1 g/L in the refold mixture. (*Compare* Appx7381 (dated April 17, 2015), *and* Appx7426 (dated June 29, 2015), *with* Appx1-12 (dated April 7, 2016).) Rather, in its subparagraph 262(*l*)(3)(B) statements, Apotex argued that claim 1 should be construed to require a 2.0 g/L or greater protein concentration in the refold mixture. (Appx7396; Appx7447.) Following the district court's construction, however, Apotex's representations were proof that its process meets the claim limitation. The protein concentration of a refold mixture does not change based on how patent claims are construed. The facts do not morph depending on what is expedient to Apotex's legal arguments.

Other than the district court's claim-construction decision (which was adverse to Apotex on this issue), Apotex provided no explanation as to why it sought to repudiate a material fact in its subparagraph 262(*l*)(3)(B) statements. At trial, Apotex's employee, Dr. Dowd, speculated as to why Apotex would report a filgrastim concentration of 0.9-1.4 g/L in its subparagraph 262(*l*)(3)(B) statements, stating that Apotex's lawyers may have seen a concentration less than 2 g/L "prominently at the start of the batch record" and "presume[d] that that would be

sufficient" to show non-infringement. (Appx3666-3667.)[3] The district court credited Dr. Dowd's testimony that the subparagraph 262(*l*)(3)(B) statements were "factually incorrect," and disregarded them as "not probative." (Appx24.)

The speculation of a witness—who by his own admission did not prepare the subparagraph 262(*l*)(3)(B) statements (*see* Appx3666-3669)—in the middle of trial cannot be sufficient to repudiate a statement of fact made in such a statement in order to frame the patent infringement issues to be litigated under the BPCIA. Accordingly, this Court should reverse the judgment of non-infringement and remand for further fact finding with the direction that the facts asserted in Apotex's subparagraph 262(*l*)(3)(B) statements should be considered. Further, Amgen respectfully submits that if Apotex is permitted to repudiate the fact statements in its subparagraph 262(*l*)(3)(B) statements, any remand should include a full opportunity for Amgen to investigate the relevant facts through discovery.

B. The District Court Erred in Not Finding that the Claimed 1 g/L Protein Concentration in the Refold Mixture Is Interchangeable with the Washed-Inclusion-Body Concentration

The district court construed "refold mixture" to mean "a mixture formed from contacting (1) the volume in which the concentration of protein is 2.0 g/L or greater with (2) the refold buffer. The refold mixture has a high protein

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3 In effect, Dr. Dowd was admitting that inclusion-body and protein concentration are viewed as one and the same thing. *See infra*.

concentration, where 'high protein concentration' is at or above about 1 g/L protein." (Appx9.) The parties did not ask the court to decide—and the court did not decide—to what, exactly, the about 1 g/L floor protein concentration refers. The reason was simple: until the trial, the parties agreed that, as a factual matter, washed-inclusion-body concentration and total protein concentration are interchangeable. This changed mid-trial when Apotex, for the first time, argued that its inclusion-body concentration is not the same as its total protein concentration because its inclusion bodies are composed of two-thirds water rather than being essentially all protein (Apotex's "water theory"). (Appx3467-3473.) This new factual dispute raised a new legal dispute: does the '138 Patent equate total protein concentration with washed-inclusion-body concentration, such that a washed-inclusion-body concentration at or above 1 g/L in the refold mixture would meet the "refold mixture" limitation of claim 1? In its post-trial submissions, Amgen argued that it does. (Appx3722; Appx3871.) The district court declined to address this argument. (Appx23-24.) In doing so, the district court erred.

The intrinsic record of the '138 Patent fully supports Amgen's position that the about 1 g/L floor protein concentration in the refold mixture of claim 1 is interchangeable with washed-inclusion-body concentration. By withholding its "water theory" until trial, Apotex deprived Amgen of the ability to make this argument during the claim-construction phase of this case (the first step of the

infringement analysis) and to fully investigate, including by experiment, whether the about 1 g/L floor protein concentration in the refold mixture is satisfied by Apotex's 0.9-1.4 g/L inclusion-body concentration (the second step of the infringement analysis). *See Innovention Toys, LLC v. MGA Entm't, Inc.*, 637 F.3d 1314, 1318-19 (Fed. Cir. 2011). This Court reviews claim-construction rulings based solely on the intrinsic record de novo. *See Teva*, 135 S. Ct. at 841. Amgen respectfully requests that this Court review the district court's determination and find, in accordance with the specification of the '138 Patent, that the about 1 g/L floor protein concentration in the refold mixture of claim 1 is interchangeable with the washed-inclusion-body concentration, and that this Court remand for further proceedings.

1. The '138 Patent Supports a Construction of Protein Concentration as Interchangeable with Washed-Inclusion-Body Concentration

The protein concentration "at or above about 1 g/L protein" in the refold mixture of claim 1 of the '138 Patent is interchangeable with the washed-inclusion-body concentration.

The '138 Patent first discusses inclusion bodies in the Background of the Invention section, explaining that a disadvantage of expressing recombinant proteins in a bacterial system is that misfolded proteins precipitate within the bacterial cells in limited solubility forms, "typically referred to as inclusion bodies." (Appx57.) Solubilization "<u>disassembles the inclusion bodies into</u>

individual protein chains with little to no structure." (*Id*. (emphasis added).) These "protein chains," i.e., the disassembled inclusion bodies, can then be "diluted into or washed with a refolding buffer that supports renaturation to a biologically active form." (*Id.*) Therefore, the patent specification contemplates that, for purposes of calculating concentration, the "protein" that is solubilized and renatured (refolded) and the "inclusion bodies" are one and the same.

The '138 Patent further uses washed inclusion bodies and protein interchangeably. For example, the specification teaches that "[w]hen the protein is disposed in inclusion bodies, the inclusion bodies can be harvested from lysed cells, washed, concentrated and refolded." (Appx61 (emphasis added).) That the specification teaches that "inclusion bodies" can be "refolded," and that the patent title and other parts of the patent refer to "refolding proteins," confirm that the patent uses the terms interchangeably.

The specification also consistently refers to protein as being expressed in the form of inclusion bodies: e.g., "the disclosed method is particularly useful for proteins expressed in bacterial expression systems, and more particularly in bacterial systems in which the protein is expressed in the form of inclusion bodies within the bacterial cell." (Appx62 (emphasis added), *accord* Appx61, Appx63.) Thus, "inclusion bodies" are a form of "protein," and not distinct from "protein." Similarly, the extrinsic evidence that the district court, in part, relied on in

construing the term "refold mixture" to include an about 1 g/L minimum protein concentration refers to protein being "expressed as" inclusion bodies. (Appx8843; Appx7632.)

Likewise, Example 1 of the '138 Patent describes the expression of recombinant <u>proteins</u> "in a limited solubility non-native form, <u>namely as inclusion bodies</u>," again equating the two terms. (Appx63.) Further, this example uses "protein" to refer to the final washed inclusion bodies. Specifically, recombinant proteins are expressed in a non-mammalian expression system as inclusion bodies, lysed, "washed multiple times," and then "centrifuge[d] to collect the <u>protein</u> in the solid fraction. The <u>final washed inclusion bodies</u> were captured and stored frozen." (*Id.* (emphasis added).)

Accordingly, a person of ordinary skill in the art would have understood that the minimum about 1 g/L concentration in the refold mixture can be either about 1 g/L protein or about 1 g/L inclusion bodies. *See Phillips*, 415 F.3d at 1315 (noting that claims "must be read in view of the specification, of which they are a part," which is usually "the single best guide to the meaning of a disputed term").

2. Before Trial, Both Parties Viewed Protein Concentration and Inclusion-Body Concentration as Being Interchangeable

Apotex's inclusion bodies are processed exactly as described in Example 1 of the '138 Patent (one of several instances where the patent uses protein and inclusion bodies interchangeably). (Appx63.) Specifically, as in Example 1,

Apotex's inclusion bodies are lysed, washed multiple times, centrifuged, and stored frozen. (Appx18.) For the refold step, an amount of such inclusion bodies is combined with Apotex's refold buffer, resulting in a concentration equivalent to 0.9-1.4 g/L in Apotex's refold mixture. (*Id.*)

Before trial, both Apotex and Amgen had viewed this 0.9-1.4 g/L inclusion-body concentration in Apotex's refold mixture as a measure of total protein concentration. For example, Apotex's expert, Dr. Robinson, had testified in deposition that the total protein concentration in Apotex's refold mixture is the 0.9-1.4 g/L inclusion-body concentration:

> Q. Yeah. Do you know what is in the inclusion bodies that are created in the Apotex or Intas process for making G-CSF?
>
> A. So we know, or I know the amount of G-CSF protein and the total protein that's in the inclusion bodies in the Apotex process.
>
> Q. What's the total <u>protein</u> amount?
>
> A. It – the total solubilized <u>inclusion bodies</u> that are fed to the refolding are <u>between point 9 and 1.4 grams per liter</u>.

(Appx9606 (emphasis added) ; *see* Appx3517-3519; *see also* Appx1282 ("the protein <u>is</u> solubilized inclusion bodies" (emphasis added)).) Dr. Robinson had also equated protein concentration with inclusion-body concentration in her opening expert report when concluding that a piece of prior art teaches "<u>protein</u>" present in a volume at a concentration of 2.0 g/L based on an <u>inclusion-body</u> concentration. (Appx10261-10262.) Likewise, Dr. Robinson conceded that in her own patent,

U.S. Patent No. 7,615,717, the term "inclusion body" "is meant to include the protein aggregate," in accordance with its ordinary meaning in the art of protein refolding. (Appx3530-3531 (discussing Appx7674).) Notably, this definition of "inclusion body" references only its protein content ("a protein aggregate") without any mention of any other compositional constituent. (*See* Appx7674-7675.) Dr. Willson had also opined that the claim requirement of a "high protein concentration" of "at or above about 1 g/L protein" is met because the "protein concentration in Apotex's refold mixture is 0.9-1.4 g/L." (Appx4123).

Importantly, before trial, the parties never disputed whether total protein concentration and inclusion-body concentration are interchangeable for purposes of satisfying the about 1 g/L floor concentration in the refold mixture. Rather, the parties first disputed the identity of the liquid to which the "2 g/L or greater" limitation of claim 1 applies. (Appx4.) Amgen argued, and the court agreed, that the "2 g/L or greater" concentration applies to the protein-containing volume, which necessarily allows for a more dilute concentration in the refold mixture—"at or above about 1 g/L protein" as construed by the court in accordance with the specification of the '138 Patent. (Appx4-5, Appx9.) After the court's claim construction, the parties subsequently disputed whether the protein concentration in the protein-containing volume and refold mixture is limited to the protein of interest or whether it also includes host-cell proteins. (Appx2466-2469.) Apotex

argued that protein concentration is limited to the concentration of the protein of interest (lower than the total protein concentration). (Appx2466-2467.) Amgen argued that protein concentration includes not only the protein of interest but also host cell proteins. (Appx2467.) Ultimately, the district court construed the term "protein" to include all protein in the refold mixture, including host cell proteins. (Appx2467-2468.)

3. By Withholding its "Water Theory" Until Trial, Apotex Deprived Amgen of the Ability To Argue During the Claim-Construction Phase of this Case that Washed-Inclusion-Body Concentration Is Interchangeable with Protein Concentration

Apotex unveiled the "water theory" for the first time in its rebuttal case, after Amgen's expert Dr. Willson had already testified. (*See* Appx3467-3473.) As Amgen stated at trial, "This theory with respect to the water was never disclosed during the expert phase of discovery. . . . Dr. Robinson has not testified about it at her deposition. She has not disclosed it in her expert reports." (Appx3467.) Rather, Amgen noted, when Dr. Robinson was asked during a deposition "What's in those inclusion bodies that's not a protein of some form," Dr. Robinson answered "There can be membranes, cell membranes, lipids," without any mention of water. (Appx3469-3470.) The district court agreed that "this opinion [Apotex's

water theory] apparently ha[d] not been disclosed by the defendant prior to this time."[4] (Appx3473.)

Apotex's "water theory" raised a new claim-construction issue with respect to the "about 1 g/L" that forms part of the district court's construction of the term "refold mixture"—does the 1 g/L refer to the washed-inclusion-body concentration? As Amgen stated, "had there been a disclosure [ ] by Apotex or by Dr. Robinson that they thought the protein concentration element was not satisfied because of water present in the inclusion bodies, [Amgen], of course, would have gone into that." (Appx3470.) This statement was true at trial, and it also applies to claim construction. By withholding its water theory until trial, Apotex deprived Amgen of the ability both to argue during the claim-construction phase of this case that washed-inclusion-body concentration is interchangeable with protein concentration, and to fully investigate what the water content of Apotex's inclusion bodies may be.

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[4] The court made this finding at trial and sustained Amgen's objection to having Dr. Robinson testify about the water theory notwithstanding Apotex's argument that at an earlier deposition, Dr. Robinson had described the output of the centrifugation step as a "wet pellet." (Appx3469.) The district court ultimately found, however, that Amgen knew or should have known that Apotex's inclusion bodies contain water based on Dr. Robinson's passing mention of a wet pellet at her deposition, although Amgen did not know—and Apotex never disclosed—how much water they allegedly contain. (Appx24.)

4. The District Court Erred in Rejecting Amgen's Post-Trial Argument that Protein Concentration and Inclusion-Body Concentration are Interchangeable and Holding that Apotex's Process Does Not Satisfy the 1 g/L Floor Concentration in the Refold Mixture

Based on Apotex's "water theory," the district court found that the 0.9-1.4 g/L inclusion-body concentration in Apotex's refold mixture "is not reliable for determining protein concentration in the refold mixture because the inclusion bodies are wet at the time of weighing and are mostly water." (Appx23.) The district court ultimately held that "Amgen did not meet its burden to show by a preponderance of the evidence that Apotex's refolding process literally uses a protein concentration in Apotex's refold mixture that is 'at or above about 1 g/L.'" (Appx33.) In so holding, the district court rejected Amgen's argument in its post-trial briefing that the floor protein concentration in the refold mixture of claim 1 is interchangeable with inclusion-body concentration. (*See* Appx3722; Appx3871.)

However, had the district court construed protein concentration and inclusion-body concentration as being interchangeable, in accordance with the specification of the '138 Patent, then the parties would have had to conduct further investigation as to whether Apotex's 0.9-1.4 g/L inclusion-body concentration in its refold mixture satisfies the "at or above about 1 g/L protein" limitation. For the foregoing reasons, Amgen respectfully requests that this Court review the district court's determination and find, in accordance with the specification of the '138 Patent, that the 1 g/L floor protein concentration in the refold mixture of claim 1 is

interchangeable with the washed-inclusion-body concentration and that this Court remand for further proceedings.

C. The District Court Failed to Properly Compare Apotex's aBLA Specifications with the Asserted Claims of the '138 Patent To Find Infringement

Under the BPCIA, the "submission" of an aBLA to FDA for approval to commercially market a biosimilar biologic product, is an act of infringement of the patents identified by the parties during the BPCIA information-exchange process. 35 U.S.C. § 271(e)(2)(C)(i); *Apotex*, 827 F.3d at 1058. Similar to the Hatch-Waxman Act (which is analogous to the BPCIA in some respects, *see Sandoz Inc.*, 794 F.3d at 1351), the ultimate infringement question, however, is determined by traditional patent-law principles.

To determine infringement, a court compares the patent claim to the aBLA specification, which is "what [the Applicant] has asked the FDA to approve as a regulatory matter." *See Sunovion*, 731 F.3d at 1278; *see also Abbott Labs. v. TorPham, Inc.*, 300 F.3d 1367, 1373 (Fed. Cir. 2002) ("Because drug manufacturers are bound by strict statutory provisions to sell only those products that comport with the ANDA's description of the drug, an ANDA specification defining a proposed generic drug in a manner that directly addresses the issue of infringement will control the infringement inquiry."). By analogy, if the aBLA applicant has asked FDA to approve a process within the scope of the claim, it

should be an infringement as a matter of law. *See Sunovion*, 731 F.3d at 1280. Manufacturing guidelines, batch records, product samples, and even certifications pledging not to infringe should not be used to overcome that infringement. *See id.* at 1278-80. This other evidence should be considered only if the aBLA is "silent" with respect to the claim limitations of the patents-in-suit. *See Meds. Co. v. Mylan Inc.*, 72 F. Supp. 3d 837, 887 (N.D. Ill. 2014) (citing *Ferring*, 764 F.3d at 1387). In such a case, the infringement question is focused on "[w]hat is likely to be sold, or, preferably, what will be sold." *Ferring*, 764 F.3d at 1388 (quoting *Glaxo, Inc. v. Novopharm, Ltd.*, 110 F.3d 1562, 1570 (Fed. Cir. 1997)).

In *Sunovion*, the generic applicant, Reddy, requested approval in its Abbreviated New Drug Application ("ANDA") to manufacture a drug product with amounts of the levorotatory isomer from 0.0-0.6%, which is within the scope of the "less than 0.25%" limitation of the asserted patent's claims. 731 F.3d at 1278. But Reddy asserted that it did not infringe because "its internal manufacturing guidelines require its generic eszopiclone products to contain at least 0.3% levorotatory isomer," and provided a certification to the district court that it would market only products containing 0.3-0.6% levorotatory isomer. *Id.* The Federal Circuit rejected Reddy's arguments, holding that Reddy's ANDA specification "seeking FDA approval for generic eszopiclone products with 0.0-0.6% levorotatory isomer mandates a finding of infringement," because "Reddy's

request for approval of levorotatory amounts from 0.0-0.6% is within the scope of the 'less than 0.25%' limitation." *Id.* In other words, Reddy's ANDA specification "would allow Reddy to sell infringing products." *Id.* (emphasis added).

Here, the district court erred by not comparing Apotex's aBLA specifications to the asserted claims of the '138 Patent. As shown below, it would be entirely possible to follow the requirements of Apotex's aBLA and have a refold mixture with a protein concentration "at or above about 1 g/L protein," in accordance with the district court's claim construction (Appx9). As in *Sunovion*, this is an infringement as a matter of law under 35 U.S.C. § 271(e)(2). *See* 731 F.3d at 1280.

Moreover, because Apotex's aBLA specifications directly address the question of protein concentration, the district court should not have considered Apotex's batch records. *See id.* at 1278-80. Indeed, consideration of Apotex's batch records was doubly improper because Apotex presented no evidence that the batch records relied on by the district court are representative of the manufacture of the product that will eventually be sold.[5] This Court reviews the district court's erroneous application of the law de novo. *See Crowley v. United States*, 398 F.3d

[5] Eighty-nine of the ninety-one batch records relied on by the district court were never produced to Amgen nor made part of the trial record.

1329, 1333 (Fed. Cir. 2005) ("Legal analysis involving the application of law to the facts is a legal question that is reviewed de novo.") (citing *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 164 (Fed. Cir. 1985)).

1. According to Apotex's aBLA Specifications, the Protein Concentration in Apotex's Refold Mixtures Can Be At or Above About 1 g/L Protein

The district court found that the "maximum concentration of total protein in Apotex's refold mixture process is limited by Apotex's aBLAs specifications to 0.708 g/L" (Appx25), failing to address that Apotex's aBLA specifications allow Apotex to run its manufacturing process in an infringing manner as to protein concentration.

First, Apotex's aBLAs expressly indicate that Apotex's refold mixture can have a protein concentration <u>greater than</u> 0.708 g/L. At trial, Apotex asserted that the maximum protein concentration in its refold mixture is 0.708 g/L. (Appx3620.) This is flawed because it incorrectly assumes that the underlying range on which this figure is calculated cannot be exceeded. Specifically, Apotex's assertion relies on the range of "4.24–11.80 mg/ml," which is the "Expected Range" for "Solubilized IB [Inclusion Body] rHu-met-GCSF Concentration" by chromatography, i.e., the filgrastim concentration in Apotex's protein-containing "volume" before it becomes part of Apotex's refold mixture measured by chromatography. (*See* Appx3617-3620; Appx4790; Appx5595;

Appx5902.) From this range, Apotex calculates that the maximum total protein concentration of its refold mixture as 0.708 g/L. (*See* Appx4790.) But the underlying 4.24-11.80 mg/ml range is characterized as a KPP (Appx5595; Appx5902), which can, by definition in the aBLAs, be exceeded. (*See* Appx6724-6725; Appx7353.) The district court erred in relying on the testimony of Apotex's fact witness Dr. Dowd that, for example, a KPP is "a specification upon which [Apotex] need[s] to maintain the process within for the batch to be acceptable" (Appx3622), because that testimony is extrinsic to—and, indeed inconsistent with—the aBLA specifications, which control the infringement analysis.

Because Apotex's aBLAs specify that the "4.24–11.80 mg/ml" KPP range can be exceeded without affecting product quality, as the district court found in denying Apotex's summary-judgment motion (Appx2471), it follows that the total protein concentration in Apotex's refold mixture can exceed 0.708 g/L as well. For example, if Apotex ran its process with a filgrastim concentration just 3 mg/mL above the KPP range specified—which Apotex is permitted to do—by Apotex's theory, the total protein concentration comes to 0.9 g/L, within "at or above <u>about</u> 1 g/L." (Appx9 (emphasis added); *see* Appx4790.) Like the ANDA specification in *Sunovion*, Apotex's aBLA specifications allow an infringement of the patent claims.

Second, as addressed at length above, Apotex's aBLA specifications call for an inclusion-body concentration of 0.9-1.4 g/L in its refold mixture. (Appx18.) The district court based its ultimate finding of non-infringement on the testimony of Apotex's witnesses that "Apotex's aBLAs specifications for the amount of inclusion bodies of 0.9 to 1.4 g/L is not reliable for determining protein concentration in the refold mixture because the inclusion bodies are wet at the time of weighing and are mostly water." (Appx23.) The district court erred because Apotex's aBLA specifications say nothing about the water—or any non-protein—content of Apotex's inclusion bodies. (*See* Appx5592-5594; Appx5900-5902.) Therefore, the testimony of Apotex's witnesses that the inclusion bodies are "mostly water" is irrelevant because it is not based on the aBLA specifications, "what [the Applicant] has asked the FDA to approve." *See Sunovion*, 731 F.3d at 1278. There is nothing in Apotex's aBLA that would render inclusion bodies consisting of pure protein non-compliant or out of specification. In addition, the 0.9-1.4 g/L inclusion-body concentration is designated as a KPP; so, it may also be exceeded. (Appx6728; Appx7359.) If Apotex's inclusion bodies are entirely or mostly protein, or if the inclusion-body concentration exceeds 0.9-1.4 g/L, the protein concentration of Apotex's refold mixture would fall within the asserted claims.

2. Consideration of Apotex's Batch Records and Testimony Regarding Those Batch Records Was Legal Error

Because Apotex's aBLA specifications directly address the protein concentration in Apotex's refold mixture, Apotex's aBLAs control the infringement inquiry, not its batch records or the testimony of its employees interpreting those batch records. *See Sunovion*, 731 F.3d at 1278-80. Apotex's aBLAs specify a KPP protein concentration measured by chromatography of 0.708 g/L in the refold mixture, which, per the aBLAs, can be exceeded to a concentration within the '138 Patent claims. This is sufficient to find an infringement because Apotex is seeking to manufacture its biosimilar products using a process that legally can fall within the scope of the '138 Patent claims. Apotex's batch records or their interpretation by an Apotex employee without first-hand knowledge of the facts recorded therein cannot save Apotex from a finding of infringement, and the district court erred in considering them.

Even if batch records could properly be considered, the batch records relied on by the district court were not shown by Apotex—nor found by the district court—to represent the manufacturing process for "[w]hat is likely to be sold, or, preferably, what will be sold" by Apotex if and when it gets FDA approval. *Ferring*, 764 F.3d at 1388. Indeed, Apotex produced only two batch records in this litigation (one for each product) (*see* Appx4250; Appx4511), while Apotex's employee, Dr. Dowd, testified at trial that Apotex's contract manufacturer ran the

manufacturing process ninety-one times. (*See* Appx3645.) No evidence was submitted regarding the additional eighty-nine records. Further, Apotex presented no evidence that either of the batches on which the district court based its decision was representative of the manufacturing process described in Apotex's aBLA, the manufacturing process used in the other eighty-nine batches, or the manufacturing process that will ultimately be used if and when Apotex receives FDA approval. Without this evidence, it was legal error for the district court to rely on these particular batch records and testimony regarding them.

CONCLUSION

For the reasons set forth above, Amgen respectfully requests that this Court reverse the district court's September 6, 2016 final judgment of no infringement of the '138 Patent, or, in the alternative, reverse and remand for further proceedings.

Dated: December 5, 2016

Respectfully submitted,

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ADDENDUM

INDEX TO ADDENDUM

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF FLORIDA

CASE NO. 15-61631-CIV-COHN/SELTZER

AMGEN, INC., and AMGEN
MANUFACTURING LIMITED,,

Plaintiffs,

v.

APOTEX INC. and APOTEX CORP.,

Defendants.
_________________________________/

CLAIM CONSTRUCTION ORDER

THIS CAUSE has come before the Court upon the parties' motions and briefs (DE [76], [77], [82], [83], [89], and [90]) for the construction of certain claim language in U.S. Patents Nos. 8,952,138 (the "'138 Patent") and 6,162,427 (the "'427 Patent").[1] The '138 patent is entitled "Refolding Proteins Using a Chemically Controlled Redox State" and was issued on February 10, 2015. The '427 patent is entitled "Combination of G-SF with a Chemotherapeutic Agent for Stem Cell Mobilization" and was issued on December 19, 2000. The patents are owned by Amgen, Inc., and Amgen Manufacturing Limited (collectively "Amgen").

Amgen develops, manufactures, and markets biologic therapy products including Neulasta (a pegylated filgrastim product) and Neupogen (a filgrastim product). Neulesta and Neupogen are, in the simplest of terms, biologic therapies which consist

[1] A third patent, Patent No. 5,824,784 (the "'784 Patent") has expired and is not considered in this Order.

of bacterial proteins that stimulate production of white blood cells in patients undergoing chemotherapy and/or stem cell transplants. The ’138 patent is directed to improved methods for refolding the proteins made in bacterial cells, allowing for industrial scale protein production. The ’427 patent provides an improved means of enhancing the mobilization of hematopoietic stem cells in patients undergoing stem cell transplants. Amgen has asserted patent claims against Apotex Inc. and Apotex Corp. (collectively “Apotex”) based upon Apotex’s filings with the U.S. Food & Drug Administration seeking approval to market biosimilar versions of Amgen’s products.

The parties dispute the meaning of several claim terms in the ’138 and ’427 patents. The Court held a hearing on February 5, 2016, at which both parties presented extensive argument. The parties agreed to rely upon the evidence and affidavits in the record and, therefore, did not present any testimony.

I. LEGAL STANDARD

The fundamental purpose of a patent is to give notice to others of the subject matter as to which the inventor claims exclusive rights. See Oakley Inc. v. Sunglass Hut Int’l, 316 F.3d 1331, 1340 (Fed. Cir. 2003). Thus, the focus of claim construction is ascertaining how one of ordinary skill in the relevant art would have understood the claim language at the time of the invention. See Phillips v. AWH Corp., 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (*en banc*).

With two exceptions not relevant here, the words used in a patent are evaluated by determining their “ordinary and customary meaning.” Id. To ascertain that meaning, the Court “looks to ‘those sources available to the public that show what a person of skill in the art would have understood disputed claim language to mean.’” Id. at 1314

(quoting Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc., 381 F.3d 1111, 1116 (Fed. Cir. 2004)). Those sources include "the words of the claims themselves, the remainder of the specification, the prosecution history, and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." Phillips, 415 F.3d at 1314 (quoting Innova, 381 F.3d at 1116).

The Court may also rely on expert testimony, which is extrinsic evidence, to determine the state of the art at the time of the invention, and how a person of ordinary skill would have understood certain terms of art at that time. Teva Pharm. USA, Inc. v. Sandoz, Inc., 135 S. Ct. 831, 841 (2015). The Court may then use these factual determinations in its legal determination of how the person of ordinary skill in the art would have understood such terms as used in the patent at issue. Id.

"It is a 'bedrock principle' of patent law that 'the claims of a patent define the invention to which the patentee is entitled the right to exclude.'" Phillips, 415 F.3d at 1312 (quoting Innova, 381 F.3d at 1115). Because the Court must examine the patent as a whole, there is a presumption that claim terms normally will be used consistently throughout a patent, such that "the usage of a term in one claim can often illuminate the meaning of the same term in other claims." Id. at 1314. Terms also must be construed in light of the entirety of the patent, not just in the context of the particular claim(s) in which they appear. Phillips, 415 F.3d at 1313. The claim language must be read in conjunction with the description in the specification. "'Usually, [the specification] is dispositive; it is the single best guide to the meaning of a disputed term.'" Id. at 1315 (quoting Vitronics Corp. v. Conceptronic, Inc., 90 F.3d 1576, 1582 (Fed. Cir. 1996)).

Even so, the Court must be careful not to import limitations from the specification's embodiment(s) into the claims. Phillips, 415 F.3d at 1319-20.

II. ANALYSIS

A. The '138 Patent

The parties identified seven disputed claim terms in the '138 Patent. The terms appear in claim 1, which reads as follows:

> 1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0g/L or greater comprising:
>
> (a) contacting the protein with a refold buffer comprising a redox component comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2mM or greater and one or more of:
>
> (i) a denaturant;
> (ii) an aggregation suppressor; and
> (iii) a protein stablizer;
>
> to form a refold mixture;
>
> (b) incubating the refold mixure; and
> (c) isolating the protein from the refold mixture.

The first term at issue is "*a protein . . . present in a volume at a concentration of 2.0g/L or greater. . . .*" Amgen's construction is: "A protein as it exists in a volume before contacting the volume with a refold buffer. The protein concentration in the volume is 2.0g/L or greater." Apotex's construction is: "a protein . . . present at a concentration of 2.0g/L or greater after dilution in a refold buffer." The sole point of difference between the parties is whether the concentration of the protein is determined before or after it is contacted with the refold buffer.

The Court agrees with Amgen that the concentration of the protein is determined *before* it is contacted with the refold buffer. This construction is consistent with the language of the claim itself, as well as the specification. *Phillips*, 415 F.3d at 1316.

Under the terms of the claim, the protein which is being refolded, is "expressed in a non-mammalian expression *and* present in a volume at a concentration of 2.0g/L or greater. . . ." DE [77-1] '138 Patent 2:52-54 (emphasis added). The specification makes clear that the protein in a volume at a concentration of 2.0g/L or greater "is contacted with a refold buffer . . . " '138 Patent 11:6-9. This is also consistent with the Background of the Invention, which states that "[u]ntil the present disclosure, these types of complex molecules could not be refolded at high concentrations, i.e., concentrations of 2.0g/L and higher, with any meaningful degree of efficiency on a small scale, and notably not on an industrial scale." DE [77-1], '138 Patent 2:17-21. Accordingly, the Court construes the claim term "*a protein . . . present in a volume at a concentration of 2.0g/L or greater. . . ."* as "A protein as it existed in a volume before contacting the volume with a refold buffer. The protein concentration in the volume is 2.0g/L or greater."

The second disputed claim term is "refold buffer." Amgen's construction is: "A preparation that supports the renaturation of protein to a biologically active form. The refold buffer comprises (1) a redox component and (2) one or more of (i) a denaturant, (ii) an aggregation suppressor, and (iii) a protein stabilizer." Apotex's construction is: "A preparation that supports the renaturation of protein to a biologically active form." Apotex argues that the components of the refold buffer are already expressly recited as limitations within claim 1, and so their inclusion in the construction of "refold buffer" is redundant and unnecessary.

The Court finds that Amgen's construction of the claim term "refold buffer" is consistent with the language of the claim and the principles of English grammar, and

that Apotex's proposed construction could lead to the creation of a refold buffer that does not contain the components required by the claim itself. This would be improper. Gillette Co. v. Energizer Holdings, Inc., 405 F.3d 1367, 1372-74 (Fed. Cir. 2005) (the word "comprising" indicates that the recited feature includes at least the listed elements). For this reason, the Court construes the term "refold buffer" as "a preparation that supports the renaturation of protein to a biologically active form. The refold buffer comprises (1) a redox component and (2) one or more of (i) a denaturant, (ii) an aggregation suppressor, and (iii) a protein stabilizer."

The third disputed claim term is "redox component." Amgen construes this term to mean "any thiol-reactive chemical or combinations of such chemicals, or solution comprising such a chemical or chemicals that facilitates a reversible thiol exchange with another thiol or the cysteine residues of a protein. The redox component comprises a final thiol-pair ratio in the range of 0.001-100 and a redox buffer strength of 2mM or greater." Apotex's construction is: "Any thiol-reactive chemical or solution comprising such a chemical that facilitates a reversible thiol exchange with another thiol or the cysteine residues of a protein."

Again, Apotex argues that Amgen's construction is redundant, because it contains terms already expressed in the claim itself. As stated above, the construction offered by Amgen is consistent with the terms of the claim and reflects the express claim language. Amgen's construction does not render any other portion of the claim superfluous. Accordingly, the Court construes the term "redox component" as "Any thiol-reactive chemical or combinations of such chemicals, or solution comprising such a chemical or chemicals that facilitates a reversible thiol exchange with another thiol or

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the cysteine residues of a protein. The redox component comprises a final thiol-pair ratio in the range of 0.001-100 and a redox buffer strength of 2mM or greater."

The fourth disputed claim term is "final thiol-pair ratio." Amgen's construction is: "Defined by the following equation:

$$\frac{[\mathit{reductant}]^2}{[\mathit{oxidant}]}$$

where the concentrations are the concentrations in the redox component." Apotex's construction is: "The relationship of the reduced and oxidized redox species used in the refold buffer as defined in Equation 1:

$$\frac{[\text{reductant}]^2}{[\text{oxidant}]}$$

where the ratio is the ratio in the refold mixture." The parties agree that the final thiol-pair ratio is based on the concentrations of the reductant and the oxidant in a solution, as defined by Equation 1 set forth at column 6, lines 23-28, but they disagree as to whether the ratio applies to the redox component (Amgen) or the refold mixture (Apotex).

Again, the plain language of the claim reveals that the redox component is comprised of a final thiol-pair ratio and one or more listed elements, combined "to form a refold mixture." This indicates that the ratio applies to the redox component and not to the refold mixture. The specification supports this conclusion as well, where it states: "After the protein has been contacted with a redox component having the recited thiol-pair ratio and redox buffer strength to form a refold mixture, the refold mixture is then incubated for a desired period of time." DE [77-1], '138 Patent 11:64-67. For this

reason, the Court constructs the term “final thiol-pair ratio” to mean “Defined by the following equation:

$$\frac{[\textit{reductant}]^2}{[\textit{oxidant}]}$$

where the concentrations are the concentrations in the redox component.”

The fifth disputed claim term is “redox buffer strength.” Amgen’s construction is: “Also called ‘buffer thiol strength,’ ‘thiol-pair buffer strength,’ or ‘thiol-pair strength,’ defined by the following equation: 2[*oxidant*] + [*reductant*] where the concentrations are the concentrations in the redox component.” Apotex’s construction is: “2[oxidant] + [reductant] where the concentrations are the concentrations in the refold mixture.” The parties agree on the equation for defining the redox buffer strength, but dispute which solution (the redox component or the refold mixture) should be used as the basis for calculating the redox buffer strength.

The Court finds that the plain language of claim 1 recites the redox buffer strength of the redox component prior to the formation of the refold mixture; the claim language is careful to say which value is measured at which stage. Adopting Apotex’s proposed construction would require the Court to re-write the claim. Additionally, Apotex’s proposed construction is contradicted by the teachings of the specification and the rebuttal declaration of Richard C. Willson, Ph.D DE [83-1]. The values of the concentrations of oxidants and reductants used in the equations in the specification are based on the volume of the redox component, and not the refold mixture. Accordingly, based upon the language of the claim and the specification, the Court construes the term “redox buffer strength” as follows: “Also called ‘buffer thiol strength,’ ‘thiol-pair

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buffer strength,' or 'thiol-pair strength,' defined by the following equation: 2[*oxidant*] + [*reductant*] where the concentrations are the concentrations in the redox component."

The sixth claim term in dispute is the term "refold mixture." Amgen's construction is: "A mixture formed from contacting (1) the volume in which the concentration of protein is 2.0g/L or greater with (2) the refold buffer. The refold mixture has a high protein concentration, where "high protein concentration" is at or above about 1g/L protein." Apotex's construction is: "A mixture formed from contacting the protein and the refold buffer." Apotex's proposed construction of the term "refold mixture" derives from its proposed construction that the term "*a protein . . . present in a volume at a concentration of 2.0g/L or greater. . . .*" is "a protein . . . present at a concentration of 2.0g/L or greater after dilution in a refold buffer." The Court has rejected that construction and rejects Apotex's construction of the term "refold mixture" as well. The language of the claim, the specification and the state of the prior art support the conclusion that the refold mixture of claim 1 of the '138 Patent would be interpreted by a person of ordinary skill in the art to have a minimum or "floor" concentration at or above about 1g/L. Thus, the Court constructs the term "refold mixture" as "a mixture formed from contacting (1) the volume in which the concentration of protein is 2.0g/L or greater with (2) the refold buffer. The refold mixture has a high protein concentration, where "high protein concentration" is at or above about 1g/L protein."

The seventh, and last, disputed claim term of the '138 Patent is the term "2mM or greater." Amgen's construction is: "No construction necessary. The term should be given its plain and ordinary meaning." Apotex's construction is: "2mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100mM."

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Apotex argues that the specification repeatedly states that the "thiol-pair buffer strength is effectively bounded at a maximum of 100mM" and, therefore, "the specification makes it abundantly clear to one skilled in the art that the patent is using the term '2mM or greater' to describe a redox buffer strength between 2mM and 100mM." DE [76, p. 19]. To the contrary, Amgen argues that the maximum of 100mM referred to in the specification is merely an embodiment, which does not impose a limitation on the language of the claim. Thus, argues Amgen, the term "2mM or greater" means what it says, with no limitation.

"It is the claims that define the metes and bounds of the patentee's invention. Phillips, 415 F.3d at 1313. The patentee is free to choose a broad term and expect to obtain the full scope of its plain and ordinary meaning unless the patentee explicitly redefines the term or disavows its full scope." Thorner v. Sony Computer Entm't Am. LLC, 669 F.3d 1362, 1367 (Fed. Cir. 2012). "[O]ne purpose for examining the specification is to determine if the patentee has limited the scope of the claims." Scimed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc., 242 F.3d 1337,1341 (Fed. Cir. 2001) (quoting Watts v. XL Sys., Inc., 232 F.3d 877, 882 (Fed. Cir. 2000)). The Court finds that the specification does, indeed, impose an upper limit of 100mM on the thiol-pair buffer strength. The Court is particularly convinced by the fact that the specification repeatedly sets forth a suggested range of redox buffer strengths, yet each time specifically limits the possible ranges, "wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100mM." Accordingly, the Court constructs the claim term ""2mM or greater" to mean "2mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100mM."

B. The ’427 Patent

The parties identify two disputed claim terms in the ’427 Patent. They are located in claims 1 and 4, which are set forth below:

> 1. A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment,
>
> comprising
>
> administering to the patient a hematopoietic stem cell mobilizing-effective amount of G-CSF; and
>
> thereafter administering to the patient a disease treating-effective amount of at least one chemotherapeutic agent.
>
> 4. The method of claim 1, wherein the at least one chemotherapeutic agent opens the enothelial barrier of the patient to render the endothelial barrier permeable for stem cells.

The first term in dispute in the ’427 Patent is “chemotherapeutic agent” as found in claim 1. Amgen’s construction is: “Exogenous substance capable of damaging or destroying microorganisms, parasites or tumor cells.” Apotex’s construction is: “Therapeutic agents which open the endothelial barrier, rendering it permeable for stem cells and/or exogenous substances suited and used to damage or destroy microorganisms, parasites or tumor cells.” The Court concludes, based upon claim 1 and the dependent claim 4, as well as the use of the term “chemotherapeutic agent” in the specification, that the term “chemotherapeutic agent” in claim 1 is not limited to therapeutic agents which open the endothelial barrier. Accordingly, the Court constructs the claim term “chemotherapeutic agent” to mean “Exogenous substance capable of damaging or destroying microorganisms, parasites or tumor cells.”

The final term in dispute in the '427 Patent is the phrase "disease treating-effective amount." Apotex argues that this term is indefinite, thus invalidating the patent. Amgen's construction is: "An amount sufficient to enhance the mobilization of stem cells for recovery from the blood for subsequent peripheral transplantation."

The specification explains that the treatment covered by the claim is for diseases that require stem cell transplantation and that the treatment "depends on the mobilization of the bone marrow stem cells . . . ." The specification also provides a dosage range for the chemotherapeutic agent of "0.05 - 100 mg/kg/day." Accordingly, the Court agrees with Amgen that "[a] person of ordinary skill in the art would understand that a dose of chemotherapeutic agent within this range, when administered after G-CSF, would be the "disease treating-effective amount" needed to achieve the goal of enhancing stem cell mobilization for recovery from blood and subsequent transplantation." DE [77], p.19. Thus, the Court constructs the term "disease treating-effective amount" to mean "[a]n amount sufficient to enhance the mobilization of stem cells for recovery from the blood for subsequent peripheral transplantation."

It is so **ORDERED.**

DONE AND ORDERED in Chambers, Fort Lauderdale, Florida, this 7th day of April, 2016.

James I. Cohn
JAMES I. COHN
United States District Judge

Copies provided to:

Counsel of record via CM/ECF

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UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF FLORIDA

CASE NO. 15-61631-CIV-COHN/SELTZER
(CONSOLIDATED WITH 15-62081-CIV-COHN/SELTZER)

AMGEN INC. and AMGEN
MANUFACTURING LIMITED,

Plaintiffs,

v.

APOTEX INC. and APOTEX CORP.,

Defendants.
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FINDINGS OF FACT AND CONCLUSIONS OF LAW

THIS CAUSE came before the Court for nonjury trial on July 11, 2016 through July 18, 2016. The parties provided the Court with revised proposed findings of fact and conclusions of law on August 18, 2016 [DE 262–65]. The Court has considered all submissions and the evidence presented at trial, and is otherwise advised in the premises.

Plaintiffs Amgen Inc. and Amgen Manufacturing Limited (collectively, "Amgen") sued Defendants Apotex Inc. and Apotex Corp. (collectively, "Apotex") under the Biologics Price Competition and Innovation Act ("BPCIA") for infringement of U.S. Patent No. 8,952,138 (the "'138 Patent"). Amgen is the owner of all rights, title, and interest in the '138 Patent, which covers a process of protein refolding. Apotex filed abbreviated Biologics License Application ("aBLA") Nos. 761026 and 761027 seeking approval from the U.S. Food & Drug Administration ("FDA") to market biosimilar versions of Amgen's Neulasta (Pegfilgrastim) and Neupogen (Filgrastim) products,

respectively. Amgen alleges that aBLA Nos. 761026 and 761027 infringe the '138 Patent under 35 U.S.C. § 271(e)(2)(C)(i), and also allege that the commercial manufacture, use, sale, offer for sale, or importation of Apotex's Pegfilgrastim and Filgrastim products will infringe the asserted claims of the '138 Patent under 35 U.S.C. § 271(a) and/or (g). Apotex alleges that the process described in its aBLAs falls outside the scope of the asserted claims of the '138 Patent and seeks a declaratory judgment of non-infringement and invalidity for lack of enablement.

For the reasons set forth below, the Court finds that Amgen has not met its burden to prove that Apotex's process for refolding Filgrastim and Pegfilgrastim infringe, either literally or under the doctrine of equivalents, each limitation of the '138 Patent. Additionally, the Court finds that Apotex has established that its process, as described in aBLA Nos. 761026 and 761027, does not infringe the '138 Patent. Having found no infringement, the Court shall dismiss without prejudice Apotex's counterclaim for invalidity. [1]

Pursuant to Federal Rule of Civil Procedure 52, the Court issues the following Findings of Fact and Conclusions of Law.

I. <u>FINDINGS OF FACT</u>

A. <u>The '138 Patent</u>

1. The '138 Patent is entitled "Refolding Proteins Using a Chemically Controlled Redox State." The '138 Patent issued on February 10, 2015, to inventors Joseph Edward Shultz, Roger Hart, and Ronald Nixon Keener, III, was assigned to

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[1] Apotex also argued at trial that its Pegfilgrastim product does not infringe the '138 Patent because pegylation of Filgrastim constitutes a "material change" to the claimed process. Because the Court finds no infringement, this argument is now moot.

Amgen Inc. The '138 Patent claims priority to Provisional U.S. Application No. 61/219,257, which was filed on June 22, 2009.

1. The Asserted Claims of the '138 Patent

2. Amgen asserted claims 1–3, 6–7, 13, 15–17, and 22–23 of the '138 Patent against Apotex. Claims 2–3, 6–7, 13, 15–17, and 22–23 depend from claim 1.

3. Claim 1 of the '138 Patent states:

> **1.** A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:
> (a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater and one or more of:
> (i) a denaturant;
> (ii) an aggregation suppressor; and
> (iii) a protein stabilizer;
> to form a refold mixture;
> (b) incubating the refold mixture; and
> (c) isolating the protein from the refold mixture.

4. Claim 2 of the '138 Patent states:

> **2.** The method of claim 1, wherein the final thiol-pair ratio is selected from the group consisting of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 and 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50.

5. Claim 3 of the '138 Patent states:

> **3.** The method of claim **1**, wherein the thiol-pair buffer strength is selected from the group consisting of greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM and 15 mM.

6. Claim 6 of the '138 Patent states:

> **6.** The method of claim 1, wherein the protein is present in the volume in a soluble form.

7. Claim 7 of the '138 Patent states:

> **7.** The method of claim 1, wherein the protein is recombinant.

8. Claim 13 of the '138 Patent states:

> **13.** The method of claim 1, wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system.

9. Claim 15 of the '138 Patent states:

> **15.** The method of claim 1, wherein the protein stabilizer is selected from the group consisting of arginine, proline, poly-ethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

10. Claim 16 of the '138 Patent states:

> **16.** The method of claim 1, wherein the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

11. Claim 17 of the '138 Patent states:

> **17.** The method of claim 1, wherein the thiol-pairs comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

12. Claim 22 of the '138 Patent states:

> **22.** The method of claim 1, wherein the isolating comprises contacting the mixture with an ion exchange separation matrix.

13. Claim 23 of the '138 Patent states:

> **23.** The method of claim 1, wherein the isolating further comprises a filtration step.

2. Claim Construction

14. In its Claim Construction Order and Sealed Omnibus Order, the Court construed certain terms of the '138 Patent as follows:

Claim Term	Court's Construction
"a protein . . . present in a volume at a concentration of 2.0 g/L or greater"	A protein as it existed in a volume before contacting the volume with a refold buffer. The protein concentration in the volume is 2.0 g/L or greater.
"refold mixture"	A mixture formed from contacting (1) the volume in which the concentration of protein is 2.0g/L or greater with (2) the refold buffer. The refold mixture has a high protein concentration, where "high protein concentration" is at or above about 1g/L protein.
"refold buffer"	A preparation that supports the renaturation of protein to a biologically active form. The refold buffer comprises (1) a redox component and (2) one or more of (i) a denaturant, (ii) an aggregation suppressor, and (iii) a protein stabilizer.
"redox component"	Any thiol-reactive chemical or combinations of such chemicals, or solution comprising such a chemical or chemicals that facilitates a reversible thiol exchange with another thiol or the cysteine residues of a protein. The redox component comprises a final thiol-pair ratio in the range of 0.001-100 and a redox buffer strength of 2mM or greater.
"final thiol-pair ratio"	Defined by the following equation: $\dfrac{[\mathit{reductant}]^2}{[\mathit{oxidant}]}$ where the concentrations are the concentrations in the redox component.
"redox buffer strength"	Also called "buffer thiol strength," "thiol-pair buffer strength," or "thiol-pair strength," defined by the following equation: $2[\mathit{oxidant}] + [\mathit{reductant}]$ where the concentrations are the concentrations in the redox component.
"2 mM or greater"	2mM or greater, wherein the redox buffer strength is effectively bounded at a maximum of 100mM.
"protein"	Any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds including but not limited to the protein of interest.

B. Apotex's Manufacturing Process

15. Apotex's refolding process for its Pegfilgrastim and Filgrastim products is described in detail in aBLA Nos. 761026 and 761027, respectively (hereinafter "Apotex's aBLAs"). Apotex's aBLAs seek FDA licensure to market biosimilar versions of Amgen's Neulasta (Pegfilgrastim) and Neupogen (Filgrastim) products, respectively.

16. Apotex's refolding process includes an "upstream" process and a "downstream" process. The end product of Apotex's upstream process is inclusion bodies. During the upstream process, Apotex performs multiple washes of the inclusion bodies with a buffer and water. Following each of these washes, the inclusion bodies are centrifuged to separate a wet "pellet" of inclusion bodies from the supernatant (liquid). The wet inclusion bodies are weighed at the conclusion of the upstream process and then frozen. The inclusion bodies remain frozen in storage until they are used in Apotex's downstream process.

17. Apotex's aBLAs specify that between 144 grams (hereinafter "grams" or "g") and 216 grams of inclusion bodies are used to begin Apotex's downstream process. In addition to specifying the wet weight of inclusion bodies carried from the upstream process into Apotex's downstream process, Apotex's aBLAs specify the amount of inclusion bodies as a concentration, as shown in the table below, which is equivalent to 0.9 to 1.4 grams per Liter (hereinafter "Liter" or "L") of Apotex's Refolding Buffer.

Table S.2.2-26: Inclusion Bodies Solubilization Operating Parameters

Operating Parameter	Operating Range	Set Point
IB amount per L of Refolding Buffer (160 L)	0.9 – 1.4 g/L	1.1 g/L
IB Solubilization Buffer volume	5.4 – 5.6 L	5.5 L
Amount of DTT added to solubilized IBs	4.44 – 5.55 g	5.00 g
Mixing time for reduction of solubilized IBs	20 – 40 min	30 min

DTT = dithiothreitol; IB = Inclusion Body

This concentration is determined by dividing the lowest and highest amounts of inclusion bodies—144 g and 216 g, respectively—by the nominal volume of the refold buffer tank, which is 160 L.

18. The first step in Apotex's downstream process is solubilization of the inclusion bodies. After the inclusion bodies are thawed in a small amount of water, they are dissolved in Apotex's solubilization buffer, resulting in a solution having a volume of 7.2 L. The solubilized inclusion bodies are then reacted with dithiothreitol ("DTT") to reduce the proteins into their primary, unfolded structure.

19. According to Apotex's aBLAs specifications, and shown in the table below, the concentration of Filgrastim in the solubilization buffer is 4.24 to 11.80 milligrams (hereinafter "milligrams" or "mg") per milliliter (hereinafter "milliliter" or "mL"), which is the same as 4.24 to 11.80 g/L.

Table S.2.2-27: Inclusion Bodies Solubilization Performance Parameters

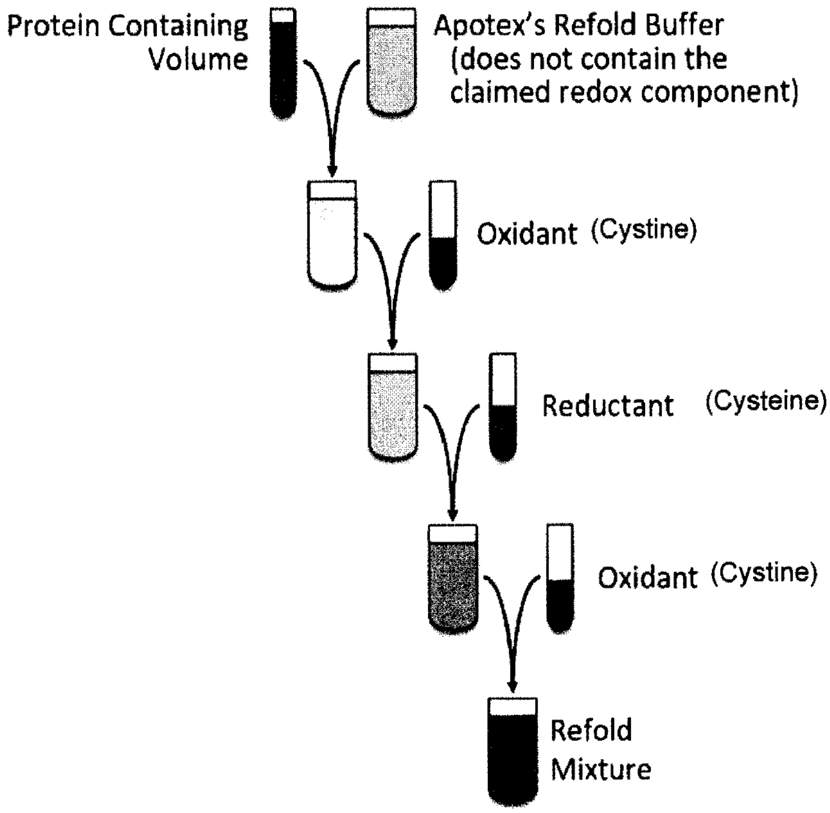
Performance Parameter	Categorization	Acceptance Criterion	Expected Range
Solubilized IB purity by UPLC M1	CPP	≥ 75%	--
Solubilized IB rHu-met-GCSF Concentration by UPLC M1	KPP	--	4.24 – 11.80 mg/mL*
Solubilized IB Endotoxin (Post-filtration)	CPP	NMT 500 EU/mg	--
Solubilized IB Bioburden (Post-filtration)	CPP	NMT 1 CFU/10 mL	--
*Based on the ranging studies that were carried out using a Design of Experiments (DoE) approach, the qualified upper limit for the concentration of protein entering the refolding unit operation is 11.8 mg/mL.			

20. Apotex's specification for the concentration of Filgrastim in the solubilization buffer limits the concentration of Filgrastim that is present in subsequent manufacturing processes. For example, the upper limit of the Filgrastim concentration in Apotex's refold mixture is 0.531 g/L. This upper limit is determined by taking the highest possible concentration of Filgrastim in the solubilization buffer—11.80 mg/mL

(or 11.80 g/L)—and multiplying by the volume of the solubilization buffer, which is 7.2 L, and then dividing by the volume of the refold mixture, which is 160 L.

21. As further shown in Table S.2.2-27: Inclusion Bodies Solubilization Performance Parameters, *supra*, Apotex's aBLAs specify that in the solubilization buffer at least 75 percent of the total protein present must be Filgrastim. This specification for the Filgrastim purity limits the amount of total protein in Apotex's refold mixture to a maximum of 0.708 g/L. This total protein amount is calculated by dividing the maximum Filgrastim concentration by 0.75 (or dividing by 75 percent).

22. Turning to Apotex's refolding process, the following schematic is illustrative:



23. The composition and quantity of ingredients in Apotex's Refolding Buffer, Cystine Solution, and Cysteine Solution are shown below in Table S.2.2-33.

Table S.2.2-33: Refolding – Solution Composition

Solution	Component	Quantity
Refolding Buffer, pH 9.0 ± 0.2, Conductivity 17.5 ± 1.5 mS/cm	Arginine base	16.8 ± 0.02 kg
	Tris base	1940.00 ± 0.02 g
	Sorbitol	8.0 ± 0.8 kg
	EDTA disodium dihydrate	118.80 ± 0.02 g
	WFI Ph. Eur., IP, USP	q.s. to 168.0 kg
Cystine Solution	Cystine	13.2 ± 3.6 g
	0.2 N Hydrochloric Acid	440 ± 4 mL
Cysteine Solution	Cysteine	2.500 ± 0.025 g
	WFI Ph. Eur., IP, USP	32.00 ± 0.32 mL

Tris = Tris (hydroxymethyl) aminomethane; WFI = Water for Injection; USP: United State Pharmacopoeia

24. The first step in Apotex's refolding process is to create Apotex's Refolding Buffer (the orange container in the schematic) and to add it to the refolding vessel. Solubilized and reduced inclusion bodies (royal blue) are then slowly added to Apotex's Refolding Buffer over 90 minutes.

25. After the solubilized and reduced inclusion bodies are added to Apotex's Refolding Buffer, the Cystine Solution (purple) and Cysteine Solution (pink) are added in a stepwise manner. According to the aBLAs, first 360 mL of the Cystine Solution (purple) is added to Apotex's Refolding Buffer to "neutralize DTT." Next, 32 mL of the Cysteine Solution (pink) is added to Apotex's Refolding Buffer to "break S-H (thiosulfide) bonds." Finally, 80 mL of Cystine Solution is added to "reduce the free S moieties so they were not available to form intramolecular disulfide bonds after refolding."

26. After the stepwise addition of the Cystine and Cysteine Solutions, Apotex incubates the refold mixture for at least 18 hours. Then, Apotex isolates the Filgrastim protein using a series of isolation steps.

27. The protein that results from Apotex's manufacturing process is Filgrastim Critical Intermediate ("Filgrastim CI"), which is both the starting material for Apotex's Filgrastim product and the critical intermediary for its Pegfilgrastim product.

C. **Apotex Does Not Infringe the Asserted Claims of the '138 Patent.**

28. As discussed in detail below, Amgen has not met its burden to show that Apotex's refolding process, as defined by Apotex's aBLAs, infringes the asserted claims of the '138 Patent, either literally or under the doctrine of equivalents. Specifically, Amgen has not established by a preponderance of the evidence that Apotex's process has: (1) a "high protein concentration" at or above about 1g/L; and (2) a redox component having a redox buffer strength of 2 to 100 mM.

29. Moreover, Apotex has shown that its manufacturing process, both as defined in its aBLAs and in practice, does not infringe the '138 Patent.

1. Apotex's Refolding Process Does Not Include a Refold Mixture Having a Protein Concentration At or Above about 1 g/L.

30. Each asserted claim of the '138 Patent requires a "refold mixture" having "a high protein concentration, where 'high protein concentration' is at or above about 1g/L protein." Amgen asserts that Apotex's refolding process literally meets this claim element, and did not allege infringement of this element under the doctrine of equivalents.

31. As discussed in detail below, Amgen did not meet its burden to show by a preponderance of the evidence that Apotex's refolding process literally uses a protein concentration in Apotex's refold mixture that is "at or above about 1 g/L." To the contrary, the Court finds that Apotex's aBLAs require a total protein concentration in

Apotex's refold mixture that is well below "at or above about 1 g/L." Therefore, the Court finds that Apotex's aBLAs do not define an infringing process.

32. The Court finds that Amgen's failure to prove that Apotex's refolding process literally infringes the asserted claims of the '138 Patent is established by: (i) the testimony of Amgen's expert Dr. Richard C. Willson, III and Apotex's experts Dr. Jason Dowd and Dr. Anne S. Robinson that Apotex's inclusion bodies are not wholly protein; (ii) Dr. Dowd's and Dr. Robinson's testimony that Apotex's aBLAs specifications for the amount of inclusion bodies of 0.9 to 1.4 g/L is not reliable for determining protein concentration in the refold mixture because the inclusion bodies are wet at the time of weighing and are mostly water; (iii) the fact that Dr. Willson's opinion that the washed inclusion bodies are almost entirely pure protein did not account for the water present in those inclusion bodies; and (iv) Amgen's lack of evidence that the actual protein concentration in Apotex's refold mixture is "at or above about 1 g/L."

33. Further, the Court finds that Apotex's non-infringement is established by: (i) Apotex's aBLAs that require a specific protein concentration range in the refold mixture that is outside the range of "at or above about 1 g/L"; and (ii) Apotex's batch records, which show that the protein concentration in the refold mixture of actual manufactured batches is outside the range of "at or above about 1 g/L."

a. Amgen did not prove that Apotex's specification for inclusion bodies defines the protein concentration in the refold mixture.

34. Amgen's theory of infringement of the protein concentration limitation requires a finding that the inclusion bodies in Apotex's downstream process are primarily pure protein. Specifically, Amgen maintains that the 0.9 to 1.4 g/L inclusion

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body concentration specification in Apotex's aBLAs is roughly equivalent to the total protein concentration.

35. The Court does not find that Apotex's inclusion bodies are substantially pure protein. In reaching this conclusion, the Court credits the testimony of Apotex's experts, Dr. Dowd and Dr. Robinson, that Apotex's inclusion bodies are composed of approximately two-thirds water at the time of weighing.

36. Amgen's theory that Apotex's inclusion body specification defines the protein concentration, as explained by Dr. Willson, does not sufficiently account for the water weight present in the inclusion bodies at the time of weighing.

37. Additionally, no evidence affirmatively shows that Apotex's centrifugation process removes water from Apotex's inclusion bodies. Dr. Willson's testimony that Apotex "pours off the liquid containing the stuff that got washed off" during centrifugation speaks to the amount of liquid on the outside of the inclusion bodies, but it does not establish how much liquid remains in them.

38. In light of Dr. Robinson's deposition testimony describing the inclusion bodies after centrifugation and at the time of weighing as a "wet pellet" and the specifications in Apotex's batch records (described in the following section), Amgen knew or should have known that the inclusion bodies contained water.

39. Apotex's pre-litigation letters to Amgen, which incorrectly equate the inclusion body concentration with protein concentration, are not probative on the issue of protein concentration. Statements in the pre-litigation letters are not binding on Apotex, and the Court credits Dr. Dowd's testimony that the statements at issue in these letters are factually incorrect.

40. Based on the above, the Court finds that Apotex's aBLAs specifications of 0.9 to 1.4 g/L merely require an amount of inclusion bodies to be used as an input in Apotex's refolding process, but do not specify the amount of protein present in those inclusion bodies. Thus, the Court finds that Amgen has failed to meet its burden to show by a preponderance of the evidence that Apotex's refolding process literally infringes the protein concentration claim limitation.

b. Apotex's aBLAs specify a protein concentration separate from an inclusion body concentration.

41. The maximum concentration of total protein in Apotex's refold mixture process is limited by Apotex's aBLAs specifications to 0.708 g/L. The Court credits the opinions and calculations of Dr. Dowd and Dr. Robinson in reaching this conclusion.

42. Apotex's aBLAs specify the concentration of Filgrastim in Apotex's solubilization buffer, and this specification limits the concentration of Filgrastim that is present in subsequent manufacturing steps.

43. As shown in Table S.2.2-27: Inclusion Bodies Solubilization Performance Parameters, *supra*, Apotex's aBLAs restrict Apotex's process from exceeding 11.80 g/L of Filgrastim in 7.2 L of solubilization buffer.

44. The upper limit of the Filgrastim concentration in Apotex's refold mixture is 0.531 g/L. This is determined by taking the highest possible concentration of Filgrastim in the solubilization buffer—11.80 g/L—and multiplying by the volume of the solubilization buffer, which is 7.2 L, and then dividing by the volume of the refold mixture, which is 160 L.

45. As further shown in Table S.2.2-27: Inclusion Bodies Solubilization Performance Parameters, *supra*, Apotex's aBLAs also specify that in the solubilization

buffer at least 75 percent of the total protein present must be Filgrastim. This specification for the Filgrastim purity effectively limits the amount of total protein in Apotex's refold mixture to a maximum of 0.708 g/L. This is calculated by dividing the maximum Filgrastim concentration in the refold mixture—0.531 g/L—by 0.75 (or dividing by 75 percent).

46. If Apotex's manufacturing process was to deviate from the amount and quantity of Filgrastim specified in the Apotex aBLAs submitted to the FDA, Apotex would be required to discard that batch. The Court credits the testimony of Dr. Dowd in reaching this conclusion.

47. Amgen cited no evidence to contradict that Apotex's aBLAs specifications limit the maximum protein concentration in Apotex's refold mixture to 0.708 g/L. Evidence that Apotex advertised that it uses a bioreactor capable of utilizing a higher protein concentration is irrelevant to the infringement inquiry because this bioreactor is used for protein synthesis and is not involved in any way in Apotex's refolding process for Filgrastim.

48. Because Apotex's aBLAs limit the amount of total protein in Apotex's refold mixture to a maximum of 0.708 g/L, the Court finds that Apotex's aBLAs specifications directly address the infringement inquiry and define a protein refolding process having a total protein concentration less than "at or above about 1 g/L protein." For these reasons, the Court finds that Apotex's refolding process does not infringe the asserted claims.

c. *Batch records show that the products that Apotex will likely market are manufactured by a non-infringing process.*

49. Apotex's batch records, which were submitted to the FDA with Apotex's aBLAs, show that Apotex's protein refolding process, in practice, has not and will not use a protein concentration in Apotex's refold mixture that is within the scope of "at or above about 1 g/L protein," as required by claim 1 of the '138 Patent.

50. Apotex's batch records document the way in which Apotex has made its Filgrastim and Pegfilgrastim products. Apotex's batch records report both the amount of wet inclusion bodies that are used to begin Apotex's refolding process, as well as the total amount of protein present in those inclusion bodies. Apotex's batch records also confirm that the total wet weight of the inclusion bodies are used to calculate the 0.9 to 1.4 g/L inclusion body concentration in the refold mixture.

51. Apotex's batch records reflect that inclusion bodies from Apotex's upstream process are weighed wet prior to being placed into cold storage for up to 90 days. That Apotex's inclusion bodies are frozen suggests that water is present with the inclusion bodies.

52. After the inclusion bodies have been solubilized, Apotex measures the total protein concentration using an optical density measurement at 280 nanometers, also referred to as "OD280." Apotex uses the OD280 measurement in the solubilization buffer to calculate the total amount of protein that was present in Apotex's inclusion bodies and records this amount in its batch records.

53. The batch records show that, in the 91 times that Apotex has run its manufacturing process, the average protein content in Apotex's inclusion bodies has been 36 percent, with the balance of Apotex's inclusion bodies—on average, 64 percent

by weight—being water. Further, in the 91 times that Apotex has run its manufacturing process, the highest protein concentration in the refold mixture has been 0.56 g/L, which is well below the claimed "at or above 1 g/L." The Court credits Dr. Dowd's testimony in reaching these findings.

54. In addition to measuring the protein concentration in the solubilization buffer, Apotex measures the protein concentration in its refold mixture using the OD280 measurement. However, this second measurement of protein concentration (taken in the refold mixture) reports an artificially higher amount of protein because cysteine and cystine are present at high concentrations, and both absorb light at 280 nanometers. Although the measurement of protein concentration in the refold mixture is not a reliable indicator of protein concentration, a clear explanation exists for the difference between the OD280 measurements from the solubilization buffer and the refold mixture. Thus, the higher OD280 measurement of protein concentration in the refold mixture does not render unreliable the OD280 measurement in the solubilization buffer.

55. For these reasons, the Court finds that Apotex's batch records provide an accurate record of Apotex's manufacturing process, which does not literally infringe any of the asserted claims of the '138 Patent.

2. Apotex's Refolding Process Does Not Include a Redox Component Having a Redox Buffer Strength of 2 to 100 mM or Its Equivalent.

56. Each of the asserted claims of the '138 Patent requires a "redox component comprising . . . a redox buffer strength of 2 mM or greater," wherein the redox buffer strength is effectively bounded at a maximum of 100 mM.

57. The claim specifies a minimum redox buffer strength because, as the Patent states, "[a]t lower redox buffer strengths, the overall system becomes much

more difficult to control." The imposition of an effective maximum redox buffer strength is to address solubility limitations.

58. Apotex's process does not literally include the claimed redox component that has an oxidant (cystine) and a reductant (cysteine) combined together outside of the refold mixture. Nor does Apotex's process literally include the claimed redox buffer strength. These conclusions are not in dispute.

59. Instead, Amgen argues that Apotex's process has (1) an equivalent redox component (2) that equivalently satisfies the buffer strength limitation.

60. The Court will assume, without deciding, that the Cysteine and Cystine Solutions added in a stepwise manner in Apotex's refolding process is the equivalent of the claimed redox component.

61. The Court does find, however, that Amgen has failed to meet its burden to prove that the hypothetical redox component in Apotex's process—the combination of Apotex's Cysteine and Cystine Solutions in a hypothetical volume—satisfies the redox buffer strength claim limitation under the doctrine of equivalents.

62. Specifically, Amgen has not proven by a preponderance of the evidence that the redox buffer strength of Apotex's hypothetical redox component is insubstantially different from the claimed redox buffer strength of 2 to 100 mM.

63. The maximum possible combined volume of Apotex's Cystine and Cysteine Solutions is 476.32 mL (444 mL of Cystine Solution plus 32.32 mL of Cysteine Solution). Thus, the maximum possible volume of Apotex's hypothetical redox component is 476.32 mL.

64. The redox buffer strength of Apotex's hypothetical redox component ranges from 214 to 340 mM.

65. Thus, Apotex's process uses a smaller volume of more concentrated redox component than is claimed in the '138 Patent to achieve its desired redox conditions.

66. According to Dr. Willson, when using a redox component with a redox buffer strength of 100 mM (within the limitation of the claim), one would need to practice the claimed method with a total volume of 1.0 L to 1.6 L of such a redox component to deliver the same number of molecules of cystine and cysteine to the refold mixture as in Apotex's process.

67. A volume of 1 to 1.6 L is two to three times greater than the volume of the hypothetical redox component.

68. The difference between a redox component in a 476.32 mL volume and a 1 to 1.6 L volume, particularly when its components are added in a stepwise manner, is substantial. The Court credits Dr. Robinson's opinion in reaching this conclusion.

69. Amgen's evidence is insufficient that simply increasing the redox component volume will serve substantially the same function in substantially the same way to achieve substantially the same result as practicing a volume with the claimed redox component strength. Dr. Willson did not specify what liquid would be used to increase the volume of the hypothetical redox component in Apotex's process to achieve the desired redox buffer strength. Dr. Willson also acknowledged that he did not know where equivalence would be lost by increasing the volume of the redox component volume. Additionally, Dr. Willson did not perform any experiments or

present any evidence that increasing the volume of the redox component would result in an insubstantial difference.

70. Additionally, Apotex's aBLAs specify the volume of each Cystine and Cysteine Solution allowed in its manufacturing process. A batch utilizing combined redox chemical solutions with a volume of 1 to 1.6 L is not possible under Apotex's aBLAs. Apotex's process does not, and cannot, meet the claim requirement of a redox buffer strength effectively bounded at a maximum of 100 mM.

II. CONCLUSIONS OF LAW

Amgen has not met its burden to prove that Apotex's process for manufacturing its Filgrastim and Pegfilgrastim products meets each and every claim limitation of the '138 Patent. Specifically, Amgen has not proven by a preponderance of the evidence that Apotex's process literally meets the protein concentration claim limitation or equivalently meets the redox buffer strength claim limitation. Thus, no finding of infringement is warranted. Apotex, however, is entitled to a judgment of non-infringement because it has proven that its manufacturing process does not satisfy at least one of the Patent's claim limitations.

"Patent infringement, whether literal or by equivalence, is an issue of fact, which the patentee must prove by a preponderance of the evidence." Siemens Med. Sols. USA, Inc. v. Saint-Gobain Ceramics & Plastics, Inc., 637 F.3d 1269, 1279 (Fed. Cir. 2011). Determining infringement requires a two-step analysis: (1) the patent claims must be construed to ascertain their scope and meaning; and (2) the claims, as properly construed, must be compared to the accused method or product. SmithKline

Diagnostics, Inc. v. Helena Labs. Corp., 859 F.2d 878, 889 (Fed. Cir. 1988). The Court previously construed the asserted claims, leaving the issue of infringement for trial.

To prove infringement, the patentee must show that an accused method meets each and every limitation of a claim, either literally or under the doctrine of equivalents. Deering Precision Instruments, L.L.C. v. Vector Distrib. Sys., Inc., 347 F.3d 1314, 1324 (Fed. Cir. 2003). "To show literal infringement of a patent, a patentee must supply sufficient evidence to prove that the accused product or process meets every element or limitation of a claim." Rohm & Haas Co. v. Brotech Corp., 127 F.3d 1089, 1092 (Fed. Cir. 1997) (citing Lemelson v. United States, 752 F.2d 1538, 1551 (Fed. Cir. 1985)). Under the doctrine of equivalents, a "process that does not literally infringe upon the express terms of a patent claim may nonetheless be found to infringe if there is 'equivalence' between the elements of the accused . . . process and the claimed elements of the patented invention." Warner-Jenkinson Co. v. Hilton Davis Chem. Co., 520 U.S. 17, 21 (1997) (citation omitted). Because Amgen has conceded that Apotex's process does not literally satisfy some limitations of claim 1 of the '138 Patent, Amgen proceeds on a theory of infringement by equivalence.

A dependent claim "incorporate[s] by reference all the limitations of the claim to which it refers." 35 U.S.C. § 112. If an independent claim is not infringed, then each corresponding dependent claim cannot be infringed. See Wahpeton Canvas Co., Inc. v. Frontier, Inc., 870 F.2d 1546, 1553 (Fed. Cir. 1989) ("It is axiomatic that dependent claims cannot be found infringed unless the claims from which they depend have been found to have been infringed . . . .").

A. Amgen Has Not Met Its Burden to Prove Literal Infringement of the Protein Concentration Claim Limitation.

Amgen did not meet its burden to show by a preponderance of the evidence that Apotex's refolding process literally uses a protein concentration in Apotex's refold mixture that is "at or above 1 g/L." Nor did Amgen proffer evidence or assert that Apotex's refolding process meets this limitation under the doctrine of equivalents.

Under the BPCIA, the "submission" of an aBLA to the FDA, which seeks approval to commercially market a biosimilar biologic product, is an act of infringement of the patents identified by the parties during the BPCIA information exchange process. 35 U.S.C. § 271(e)(2)(C)(i); Amgen Inc. v. Apotex Inc., 2016 WL 3606770, at *4. Similar to the Hatch-Waxman Act (which is analogous to the BPCIA in some respects, see Amgen Inc. v. Sandoz Inc., 794 F.3d 1347, 1351 (Fed. Cir. 2015)), the ultimate infringement question, however, is determined by traditional patent law principles. See Sunovion Pharmaceuticals, Inc. v. Teva Pharmaceuticals USA, Inc., 731 F.3d 1271, 1278 (Fed. Cir. 2013). If the process that an aBLA applicant is asking the FDA to approve falls within the scope of an asserted patent claim, a judgment of infringement must necessarily ensue. Id.

To determine infringement, a court compares the patent claim to the aBLAs specification, which is "what [the applicant] has asked the FDA to approve as a regulatory matter." Id.; see also Abbott Labs. v. TorPham, Inc., 300 F.3d 1367, 1373 (Fed. Cir. 2002) ("Because drug manufacturers are bound by strict statutory provisions to sell only those products that comport with the ANDA's description of the drug, an ANDA specification defining a proposed generic drug in a manner that directly addresses the issue of infringement will control the infringement inquiry."). If the aBLA

applicant has asked the FDA to approve a process within the scope of the claim, it is an infringement as a matter of law. See Sunovion, 731 F.3d at 1280. Manufacturing guidelines, batch records, product samples, and certifications pledging not to infringe cannot be used to overcome that infringement. See id. at 1278–80. This other evidence is considered only if the aBLA is "silent" with respect to the claim limitations of the patents-in-suit. See Meds. Co. v. Mylan Inc., 72 F. Supp. 3d 837, 887 (N.D. Ill. 2014) (citing Ferring B.V. v. Watson Labs., Inc.-Fla., 764 F.3d 1382, 1387 (Fed. Cir. 2014)). It is the burden of the patentee to prove by a preponderance of the evidence that the alleged infringer will likely market an infringing product, and that burden is never shifted to the alleged infringer. See Glaxo, Inc. v. Novopharm, Ltd., 110 F.3d 1562, 1568–70 (Fed. Cir. 1997).

Here, Amgen asserts that Apotex's aBLAs speak directly to the issue of infringement because Apotex's aBLAs contain process specifications for inclusion bodies. However, Amgen has not established that Apotex's specification for inclusion bodies defines a protein concentration in the refold mixture. Instead, the Court finds extensive evidence that Apotex's inclusion bodies are wet at the time they are weighed and are on average about two-thirds water. Further, whether Apotex refers to the inclusion bodies as a "pellet" or a "paste," does not change the fact that water constitutes the majority of Apotex's inclusion bodies at the time of weighing. Nor is this finding changed because Apotex's pre-litigation letters under 42 U.S.C. § 262(l)(3)(B) incorrectly referred to the inclusion body concentration as the protein concentration. These letters were not part of Apotex's aBLAs, were never filed with the FDA, do not impact the process and product approved by the FDA, and are not controlling. See

Takeda Chem. Indus., Ltd. v. Mylan Labs., Inc., 549 F.3d 1381, 1390–91 (Fed. Cir. 2008) ("It is clear from the district court's opinion that it . . . [did not] limit the filers to the theories raised in their certification letters.").

Apotex, however, did prove that its aBLAs specify a protein concentration separate from an inclusion body concentration. Based on the highest allowable Filgrastim concentration required by Apotex's aBLAs, the maximum total protein concentration allowable in Apotex's refold mixture is restricted at 0.708 g/L. Therefore, Apotex's aBLAs specifications directly show that the total protein concentration in Apotex's refold mixture is outside the "at or above about 1 g/L protein" range required by the Court's construction of the claim element "refold mixture." Amgen cited no relevant evidence contradicting that Apotex's aBLAs specifications effectively limit the maximum protein concentration in Apotex's refold mixture to 0.708 g/L. As a result, the Court finds that Apotex's aBLAs specifications directly address the infringement inquiry and define a protein refolding process having a total protein concentration less than "at or above about 1 g/L protein." See Sunovion, 731 F.3d at 1279–80 (citing Bayer, 212 F.3d at 1250) ("In Bayer, we upheld a summary judgment of no literal infringement because the generic manufacturer's ANDA specification itself required that the proposed product have a specific surface area outside of the range claimed by the innovator's asserted patent."). For at least these reasons, the Court finds that Apotex's refolding process does not infringe the asserted claims.

Furthermore, even if Apotex's aBLAs had been silent on the issue of protein concentration, Apotex's batch records show that the drug products it intends to market are manufactured by a non-infringing process. In the 91 times that Apotex has run its

manufacturing process, the highest protein concentration in the refold mixture has been 0.56 g/L, which is well below the claimed "at or above about 1 g/L" limitation. Apotex submitted its batch records, which include an outline for each step in the manufacturing process with operating parameters, to the FDA along with the aBLAs, and there is no evidence that the FDA has questioned the accuracy of Apotex's measurements. Thus, Apotex's batch records support a finding that judgment of non-infringement is proper because Apotex's refolding process for the drugs it intends to market does not infringe any asserted claim of the '138 Patent under 35 U.S.C. § 271(e)(2). <u>See</u> <u>Glaxo</u>, 110 F.3d at 1568–70.

B. Amgen Has Not Met Its Burden to Prove Equivalent <u>Infringement of the Redox Buffer Strength Claim Limitation.</u>

Amgen has not proven that Apotex's protein refolding process infringes the redox buffer strength claim limitation of the '138 Patent under the doctrine of equivalents. A patent is infringed under the doctrine of equivalents if the difference(s) between a claim limitation and the corresponding element in the accused process is "insubstantial" ("insubstantial differences" test). <u>See</u> <u>Warner-Jenkinson</u>, 520 U.S. at 39–40 (1997). Alternatively, an element in the accused process is equivalent to a claim limitation only if it performs substantially the same function, in substantially the same way, to yield substantially the same result ("function-way-result" test). <u>See</u> <u>id.</u> at 38–40 (citing <u>Union Paper-Bag Mach. Co. v. Murphy</u>, 97 U.S. 120, 125 (1877)). Which test to apply depends on the facts of the case, because "[d]ifferent linguistic frameworks may be more suitable to different cases, depending on their particular facts." <u>Warner-Jenkinson</u>, 520 U.S. at 40.

"What constitutes equivalency must be determined against the context of the patent, the prior art, and the particular circumstances of the case." Graver Tank & Mfg. Co. v. Linde Air Products Co., 339 U.S. 605, 609 (1950). The doctrine of equivalents "must be applied to individual elements of the claim, not to the invention as a whole." Warner-Jenkinson, 520 U.S. at 29. The patentee must demonstrate that a claim element is found equivalently in the accused product or process by a preponderance of the evidence. Id., 520 U.S. at 37. The equivalence must have been known at the time of the alleged infringement to a person having ordinary skill in the art. Graver Tank, 339 U.S. at 609.

In addition to Amgen's failure to prove that Apotex's protein refolding process literally satisfies the protein concentration limitation, Amgen has not established that Apotex's process equivalently satisfies the limitation of a "redox buffer strength of 2mM or greater." Assuming without deciding that Apotex's hypothetical redox component is equivalent to the claimed redox component, the redox buffer strength of this hypothetical redox component would be 214 to 340 mM. This value is more than two to three times greater than the maximum redox buffer strength of 100 mM permitted under the Court's claim construction. Amgen has established neither that this is an insubstantial difference nor that a redox buffer strength of 214 to 340 mM performs substantially the same function, in substantially the same way, to yield substantially the same result as the claimed redox buffer strength in the redox component.

The relevant inquiry is whether a redox component with a redox buffer strength of 214 to 340 mM is insubstantially different from a redox component with a redox buffer strength of 100 mM. To demonstrate equivalence, Dr. Willson adjusted the volume of

the hypothetical redox component from approximately 472 mL to 1.0 to 1.6 L, adding an unspecified liquid, in an effort to make the redox buffer strength of Apotex's hypothetical redox component meet the redox buffer strength claim limitation. In other words, Amgen attempts to show equivalence by significantly altering Apotex's process. This cannot be done. Apotex is bound by the specifications in its aBLAs and cannot, in practice, increase the volume of its redox component to a volume of 1.0 to 1.6 L without facing serious legal penalties. Moreover, adjusting the volume of the hypothetical redox component to reach a desired redox buffer strength that is not actually utilized in Apotex's process renders meaningless the maximum limit of 100 mM because one could simply adjust the volume of any redox component with a redox component greater than 100 mM to make it fall within the claimed limitation. See Warner-Jenkinson, 520 U.S. at 29 ("It is important to ensure that the application of the doctrine [of equivalents], even as to an individual element, is not allowed such broad play as to effectively eliminate that element in its entirety.").

For all of the reasons above, judgment of no infringement under the doctrine of equivalents is appropriate because Amgen has failed to prove by a preponderance of the evidence that a redox buffer strength 214 to 340 mM in the redox component is insubstantially different from the claimed redox buffer strength. Because the process defined in Apotex's aBLAs does not infringe claim 1, dependent claims 2, 3, 6, 7, 13, 15, 16, 17, 22, and 23 that depend from claim 1 similarly are not infringed. See Teledyne McCormick Selph, 558 F.2d at 1004.

C. **Apotex's Invalidity Counterclaim Shall Be Dismissed.**

Having found that the manufacturing process defined in Apotex's aBLAs does not infringe the '138 Patent, the Court declines to render an opinion as to whether the '138 Patent is invalid for lack of enablement. The Federal Circuit has indicated that "a district court can dismiss an invalidity counterclaim when it finds noninfringement or dismisses an infringement claim with prejudice." AstraZeneca LP v. Breath Ltd., 542 F. App'x 971, 981 (Fed. Cir. 2013), as amended on reh'g in part (Dec. 12, 2013) (citing Liquid Dynamics Corp. v. Vaughan Co., Inc., 355 F.3d 1361, 1371 (Fed. Cir. 2004) ("A district court judge faced with an invalidity counterclaim challenging a patent that it concludes was not infringed may either hear the claim or dismiss it without prejudice, subject to review only for abuse of discretion."); Nystrom v. TREX Co., Inc., 339 F.3d 1347, 1351 & n. * (Fed. Cir. 2003) ("[T]he district court could have dismissed the counterclaim without prejudice (either with or without a finding that the counterclaim was moot) following the grant of summary judgment of non-infringement."); Phonometrics, Inc. v. N. Telecom Inc., 133 F.3d 1459, 1468 (Fed. Cir. 1998) ("We have previously held that a district court has discretion to dismiss a counterclaim alleging that a patent is invalid as moot where it finds no infringement.")). "Where . . . non-infringement is clear and invalidity is not plainly evident, it is appropriate to treat only the infringement issue." Leesona Corp. v. United States, 530 F.2d 896, 906 n.9 (Ct. Cl. 1976) (citation omitted). Even after the invalidity counterclaim has been tried, the district court may dismiss the invalidity counterclaim without prejudice where "the non-infringement judgment firmly and clearly resolves the case, and [the defendant] has not shown how a judgment of

invalidity would provide any additional benefit." AstraZeneca LP, 542 F. App'x at 981–82.

Here, a judgment of Apotex's non-infringement firmly and clearly resolves this case. Apotex has not shown how a finding of invalidity of the '138 Patent would provide any additional benefit beyond a judgment of non-infringement. Moreover, unlike Apotex's non-infringement, the issue of invalidity is not plainly evident to the Court based on the evidence presented at trial. Accordingly, the Court defers judgment on the issue of invalidity of the '138 Patent and will dismiss the invalidity counterclaim without prejudice.

D. This Is Not an Unusual Case Warranting an Attorneys' Fee Award.

Under 35 U.S.C. § 271(e)(4), a court may award reasonable attorneys' fees under 35 U.S.C. § 285 to the prevailing party in exceptional cases. An exceptional case is "simply one that stands out from others with respect to the substantive strength of a party's litigating position (considering both the governing law and the facts of the case) or the unreasonable manner in which the case was litigated." Octane Fitness, LLC v. ICON Health & Fitness, Inc., 134 S. Ct. 1749, 1756 (2014); see also ILOR, LLC v. Google, Inc., 631 F.3d 1372, 1380 (Fed. Cir. 2011) (reversing district court finding that case was exceptional where neither plain language of claim, specification, nor prosecution history showed that patentee's claim construction "was so unreasonable that no reasonable litigant could believe it would succeed"). Attorneys' fees are limited to exceptional cases "in order to avoid penalizing a party for merely defending or prosecuting a lawsuit, and are awarded to avoid a gross injustice." Revlon, Inc. v.

Carson Prod. Co., 803 F.2d 676, 679 (Fed. Cir. 1986) (internal citations and quotations omitted).

Determining whether a case is "exceptional" is a case-by-case exercise that should consider the totality of the circumstances. Id. "The determination whether a case is 'exceptional' is indisputably committed to the discretion of the district court." Lumen View Tech. LLC v. Findthebest.com, Inc., 811 F.3d 479, 482 (Fed. Cir. 2016) (citing Highmark Inc. v. Allcare Health Mgmt. Sys., Inc., 134 S. Ct. 1744, 1749 (2014)).

The Court does not find this case "exceptional." Amgen's actions in asserting its patent rights were reasonable. The Court has no reason to doubt that Amgen brought this case upon a good faith belief that Apotex's process practices each claim of the '138 Patent. The substantive strength of Amgen's litigating position certainly was not so weak that no reasonable litigant would think its claims could succeed. To the contrary, Amgen advanced a cogent argument for a finding of infringement, and it should not be penalized simply because the Court found Apotex's evidence and arguments more convincing. Furthermore, Amgen litigated this case in a reasonable and professional manner. No manifest injustice will result if attorneys' fees are not awarded.

III. CONCLUSION

For the foregoing reasons, it is **ORDERED AND ADJUDGED** that a separate Final Judgment will be entered in favor of Defendants Apotex Inc. and Apotex Corp. and against Plaintiffs Amgen Inc. and Amgen Manufacturing Limited on the issue of infringement consistent with the Findings of Fact and Conclusions of Law herein.

DONE AND ORDERED in Chambers at Fort Lauderdale, Broward County,

Florida, this 6th day of September, 2016.

JAMES I. COHN
United States District Judge

Copies provided to:
Counsel of record via CM/ECF

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF FLORIDA

CASE NO. 15-61631-CIV-COHN/SELTZER
(CONSOLIDATED WITH 15-62081-CIV-COHN/SELTZER)

AMGEN INC. and AMGEN
MANUFACTURING LIMITED,

Plaintiffs,

v.

APOTEX INC. and APOTEX CORP.,

Defendants.
_______________________________/

FINAL JUDGMENT

THIS CAUSE came before the Court in a nonjury trial on July 11, 2016 through July 18, 2016, after which the Court found that Defendants Apotex Inc. and Apotex Corp. (collectively, "Apotex") have not infringed claims 1–3, 6, 7, 13, 15–17, and 22–23 (the "Asserted Claims") of U.S. Patent No. 8,952,138 ("the '138 Patent") held by Plaintiffs Amgen Inc. and Amgen Manufacturing Limited (collectively, "Amgen"). The Court entered separately its Findings of Fact and Conclusions of Law [DE 267]. Pursuant to Federal Rule of Civil Procedure 58, it is hereby

ORDERED AND ADJUDGED as follows:

1. Judgment is entered in favor of Apotex and against Amgen on:
   a. Amgen's claims of infringement under 35 U.S.C. § 271(e)(2)(C)(i): Amgen's First Count in each of Amgen's Complaints in this consolidated action [DE 1 in Case No. 15-62081; DE 1 in Case No. 15-61631];

b. Amgen's claims seeking declaratory judgments of infringement under 35 U.S.C. § 271(g): Amgen's Second Count of Amgen's Filgrastim Complaint [DE 1 in Case No. 15-62081] and Amgen's Third Count of Amgen's Pegfilgrastim Complaint [DE 1 in Case No. 15-61631]; and

c. Apotex's counterclaims regarding non-infringement of the '138 Patent: First Counterclaim (Declaratory Judgment of Non-Infringement of the '138 Patent) in each of Apotex's Answers, Affirmative Defenses, and Counterclaims in this consolidated action [DE 47 (incorporating DE 35) in Case No. 15-61631; DE 64 in Case No. 15-61631].

2. Judgment is entered in favor of Amgen and against Apotex on the following issues based on the Court's previous decision on December 9, 2015 [DE 71], the relevant part of which was affirmed by the United States Court of Appeals for the Federal Circuit on July 5, 2016 [DE 259]:

   a. Amgen's claims for Declaratory Judgment that Apotex's Notice of Commercial Marketing Violates 42 U.S.C. § 262(l)(8)(A): Amgen's Fourth Count in each of Amgen's Complaints in this consolidated action [DE 1 in Case No. 15-62081; DE 1 in Case No. 15-61631];

   b. Apotex's counterclaims for Declaratory Judgment that Subsection (k) Applicants Who Have Complied with 42 U.S.C. § 262(l)(2)(A) May Elect Not to Provide Notice of Commercial Marketing to the Reference Product Sponsor, Subject to the Consequences Set Forth in 42 U.S.C. § 262(l)(9)(B): Apotex's Fifth Counterclaim in Apotex's Pegfilgrastim Counterclaims [DE 47 (incorporating DE 35) in Case No. 15-61631] and

Apotex's second Seventh Counterclaim in Apotex's Filgrastim Counterclaims [DE 64 in Case No. 15-61631]; and

c. Apotex's counterclaims for Declaratory Judgment of No Injunctive Relief Under BPCIA: Apotex's Sixth Counterclaim in Apotex's Pegfilgrastim Counterclaims [DE 47 (incorporating DE 35) in Case No. 15-61631] and Apotex's Eighth Counterclaim in Apotex's Filgrastim Counterclaims [DE 64 in Case No. 15-61631].

3. In addition, consistent with the Court's grant of a preliminary injunction in favor of Amgen on December 9, 2015 [DE 71], affirmed by the United States Court of Appeals for the Federal Circuit on July 5, 2016 [DE 259], permanent injunctive relief is appropriate. If the FDA approves Apotex's aBLA for its Pegfilgrastim Product, Apotex must provide Amgen with at least 180 days' notice before the date of the first commercial marketing of the biological product approved by the FDA. 42 U.S.C. § 262(l)(8)(A). Apotex and those acting in concert with it are enjoined from any commercial marketing of Apotex's Pegfilgrastim Product, including selling that product or offering it for sale for use in the United States, until Apotex gives Amgen proper notice, at least 180 days before first commercial marketing but not before Apotex's Pegfilgrastim Product is licensed by the FDA, and the 180-day notice period is exhausted.

4. Likewise, if the FDA approves Apotex's aBLA for its Filgrastim Product, Apotex must provide Amgen with at least 180 days' notice before the date of the first commercial marketing of the biological product approved by the FDA. 42 U.S.C. § 262(l)(8)(A). Apotex and those acting in concert with it are enjoined from any

commercial marketing of Apotex's Filgrastim Product, including selling that product or offering it for sale for use in the United States, until Apotex gives Amgen proper notice, at least 180 days before first commercial marketing but not before Apotex's Filgrastim Product is licensed by the FDA, and the 180-day notice period is exhausted.

5. In addition, judgment is hereby entered in favor of Amgen and against Apotex on Apotex's counterclaim for Declaratory Judgment of Unenforceability of the '138 Patent for Patent Misuse: Apotex's Fourth Counterclaim in Apotex's Pegfilgrastim Counterclaims [DE 47 (incorporating DE 35) in Case No. 15-61631] and Apotex's Sixth Counterclaim in Apotex's Filgrastim Counterclaims [DE 64 in Case No. 15-61631]. Apotex neither provided evidence on this counterclaim at trial nor identified it as a trial issue in the parties' Joint Pretrial Stipulation [DE 217].
6. Apotex's counterclaims regarding invalidity of the '138 Patent for lack of enablement[1]—Apotex's Fifth Affirmative Defense (Invalidity) and Second Counterclaim (Declaratory Judgment on Invalidity of the '138 Patent) in each of Apotex's Answers, Affirmative Defenses, and Counterclaims in this consolidated action [DE 47 (incorporating DE 35) in Case No. 15-61631; DE 64 in Case No. 15-61631] —are **DISMISSED without prejudice**.
7. The Clerk of Court is directed to **CLOSE** these cases and **DENY as moot** any pending motions.

[1] The Court previously entered judgment in favor of Amgen and against Apotex on Apotex's Fifth Affirmative Defense and Second Counterclaim in each of Apotex's Answers, Affirmative Defenses, and Counterclaims in this consolidation action [DE 47 (incorporating DE 35) in Case No. 15-61631; DE 64 in Case No. 15-61631] solely with respect to (i) anticipation, (ii) lack of written description, (iii) indefiniteness, and (iv) obviousness. See DE 245.

DONE AND ORDERED in Chambers at Fort Lauderdale, Broward County, Florida, this 6th day of September, 2016.

JAMES I. COHN
United States District Judge

Copies provided to:
Counsel of record via CM/ECF

US008952138B2

(12) **United States Patent**
Shultz et al.

(10) **Patent No.: US 8,952,138 B2**
(45) **Date of Patent: *Feb. 10, 2015**

(54) **REFOLDING PROTEINS USING A CHEMICALLY CONTROLLED REDOX STATE**

(75) Inventors: **Joseph Edward Shultz**, Santa Rosa Valley, CA (US); **Roger Hart**, Loveland, CO (US); **Ronald Nixon Keener, III**, Newbury Park, CA (US)

(73) Assignee: **Amgen Inc.**, Thousand Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 403 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/820,087**

(22) Filed: **Jun. 21, 2010**

(65) **Prior Publication Data**

US 2010/0324269 A1 Dec. 23, 2010

Related U.S. Application Data

(60) Provisional application No. 61/219,257, filed on Jun. 22, 2009.

(51) **Int. Cl.**
C07K 1/22 (2006.01)
C07K 1/113 (2006.01)

(52) **U.S. Cl.**
CPC ***C07K 1/1133*** (2013.01)
USPC **530/413**

(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Yunsoo Kim
(74) *Attorney, Agent, or Firm* — David B. Ran

(57) **ABSTRACT**

A method of refolding proteins expressed in non-mammalian cells present in concentrations of 2.0 g/L or higher is disclosed. The method comprises identifying the thiol pair ratio and the redox buffer strength to achieve conditions under which efficient folding at concentrations of 2.0 g/L or higher is achieved and can be employed over a range of volumes, including commercial scale.

24 Claims, 8 Drawing Sheets

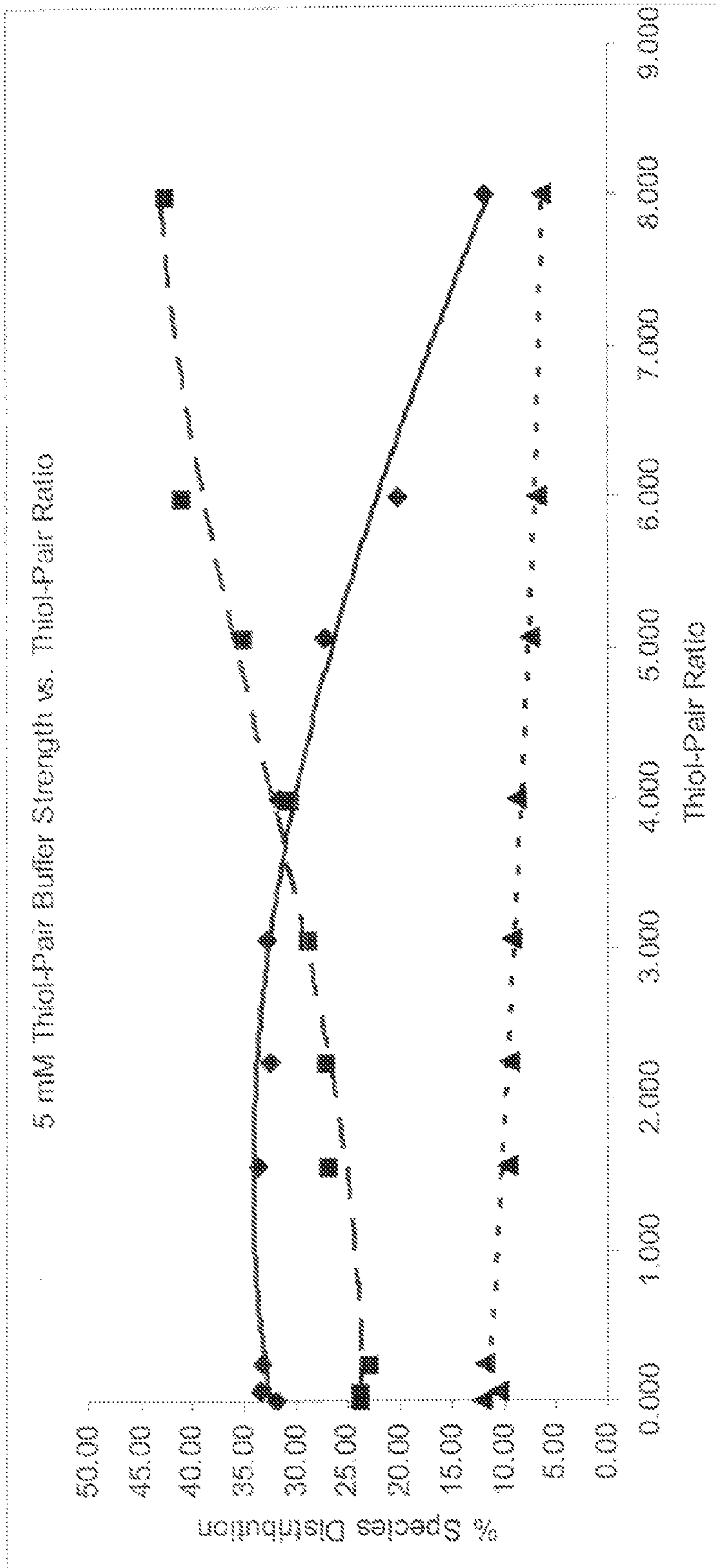
5 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
% Species Distribution
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.000
1.000
2.000
3.000
4.000
5.000
6.000
7.000
8.000
9.000
Thiol-Pair Ratio

Figure 1a

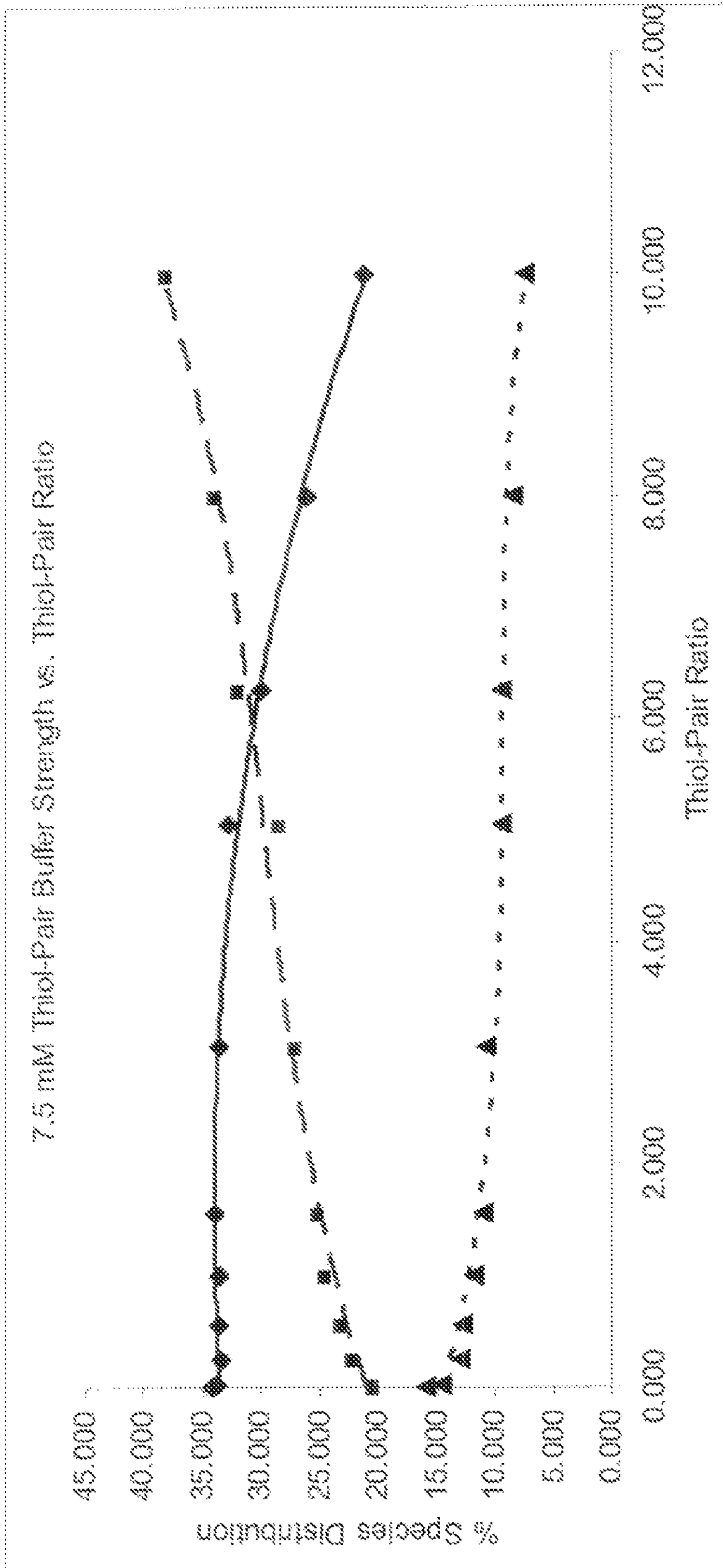
7.5 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
% Species Distribution
45.000
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
0.000
2.000
4.000
6.000
8.000
10.000
12.000
Thiol-Pair Ratio

Figure 1b

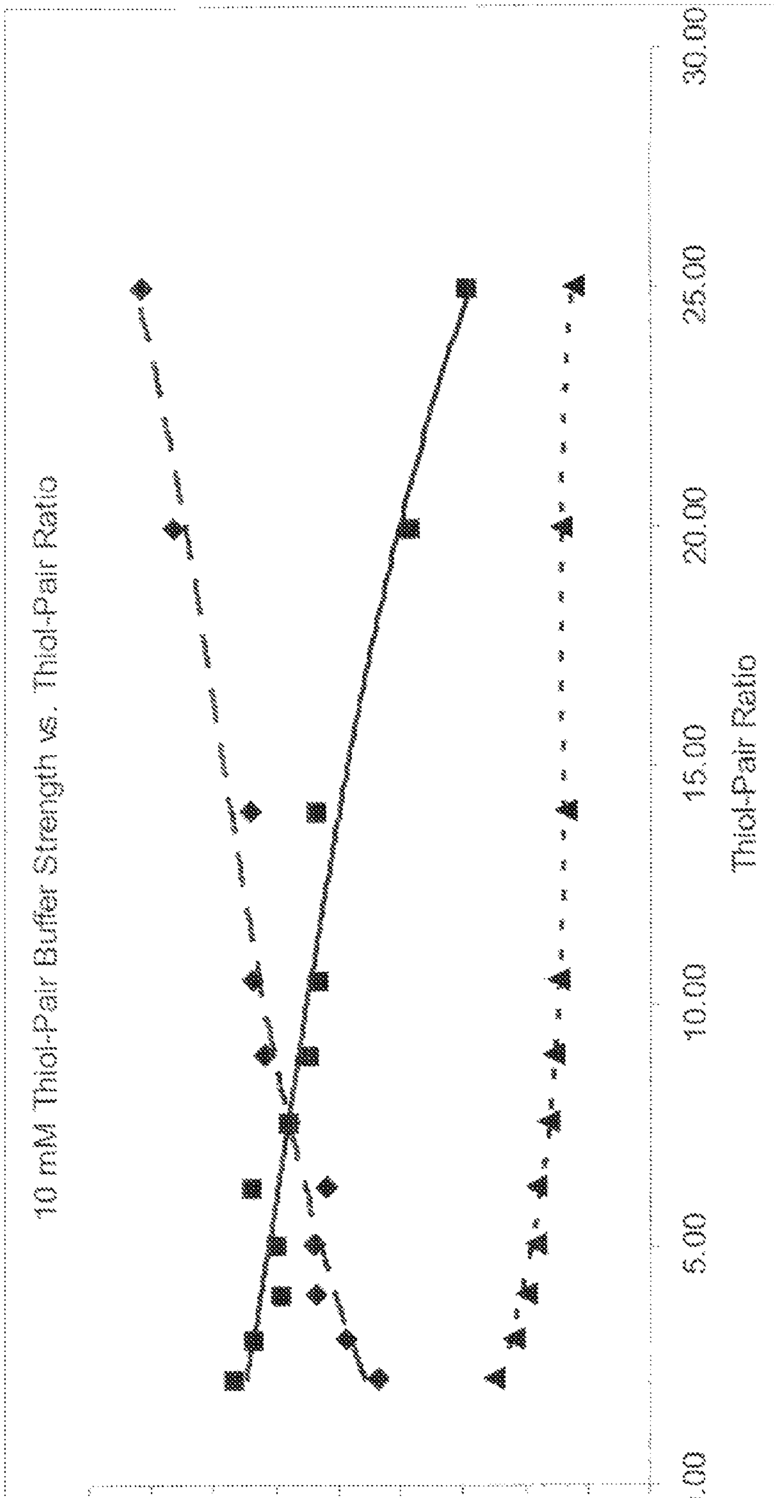
10 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
% Species Distribution
45.000
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Thiol-Pair Ratio

Figure 1c

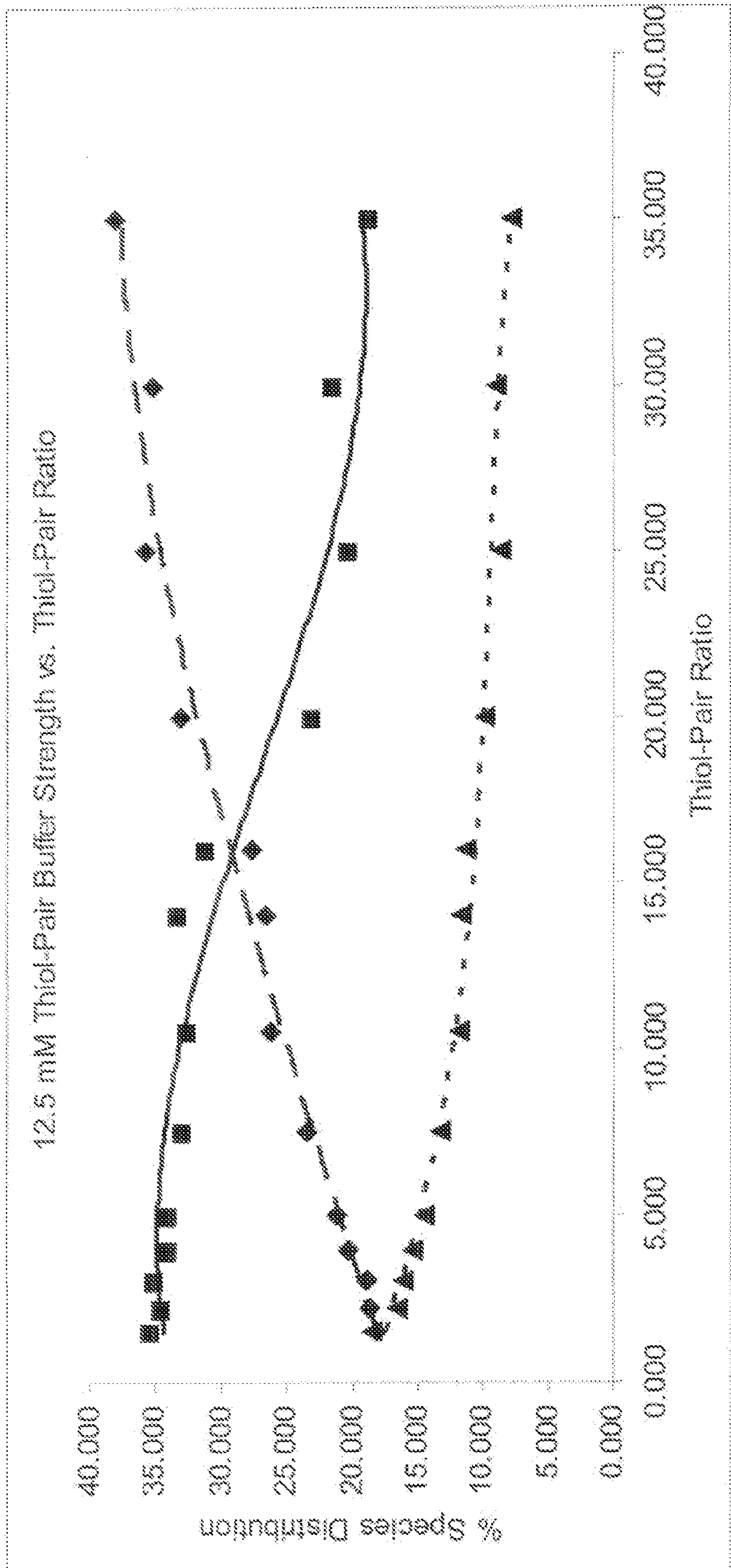
12.5 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
% Species Distribution
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Thiol-Pair Ratio
0.000
5.000
10.000
15.000
20.000
25.000
30.000
35.000
40.000

Figure 1d

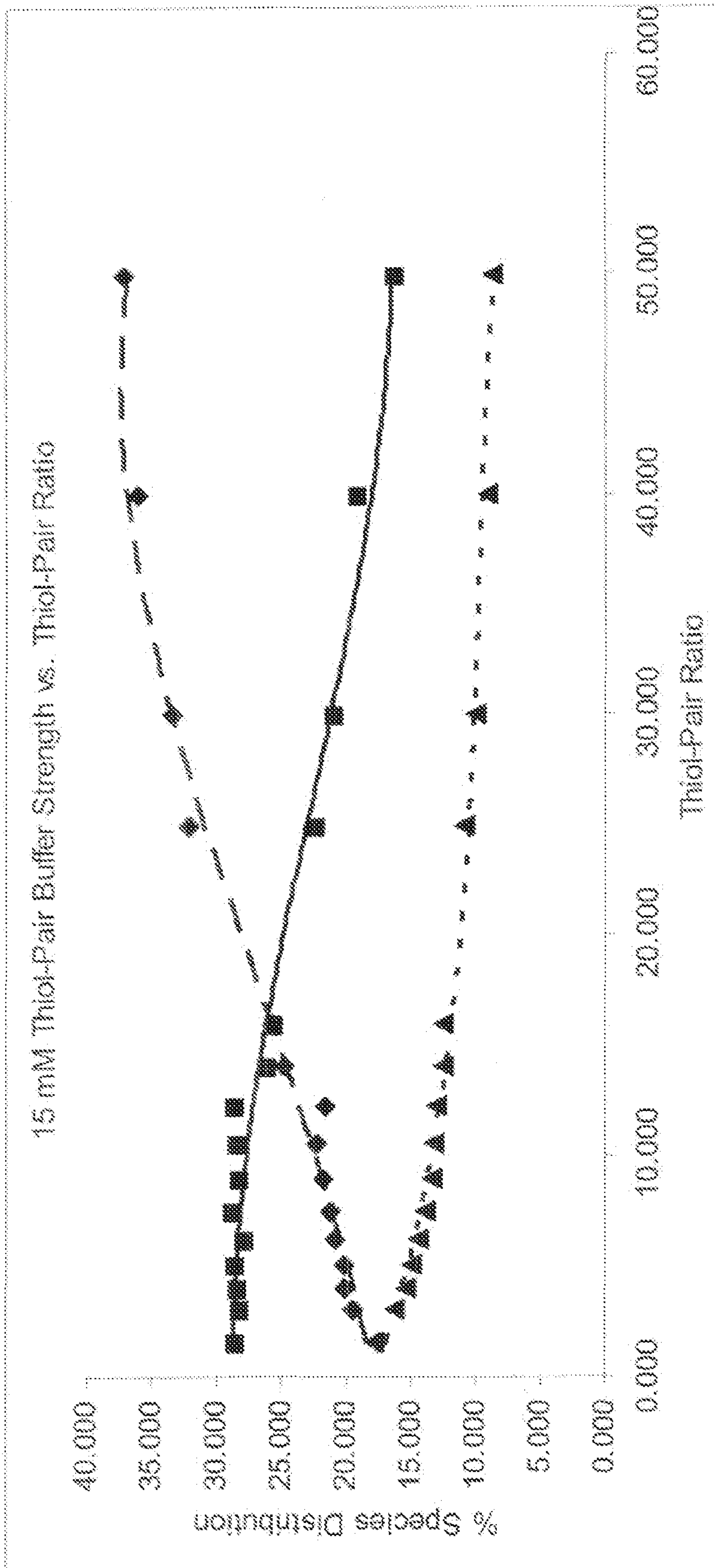
15 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
% Species Distribution
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
0.000
10.000
20.000
30.000
40.000
50.000
60.000
Thiol-Pair Ratio

Figure 1e

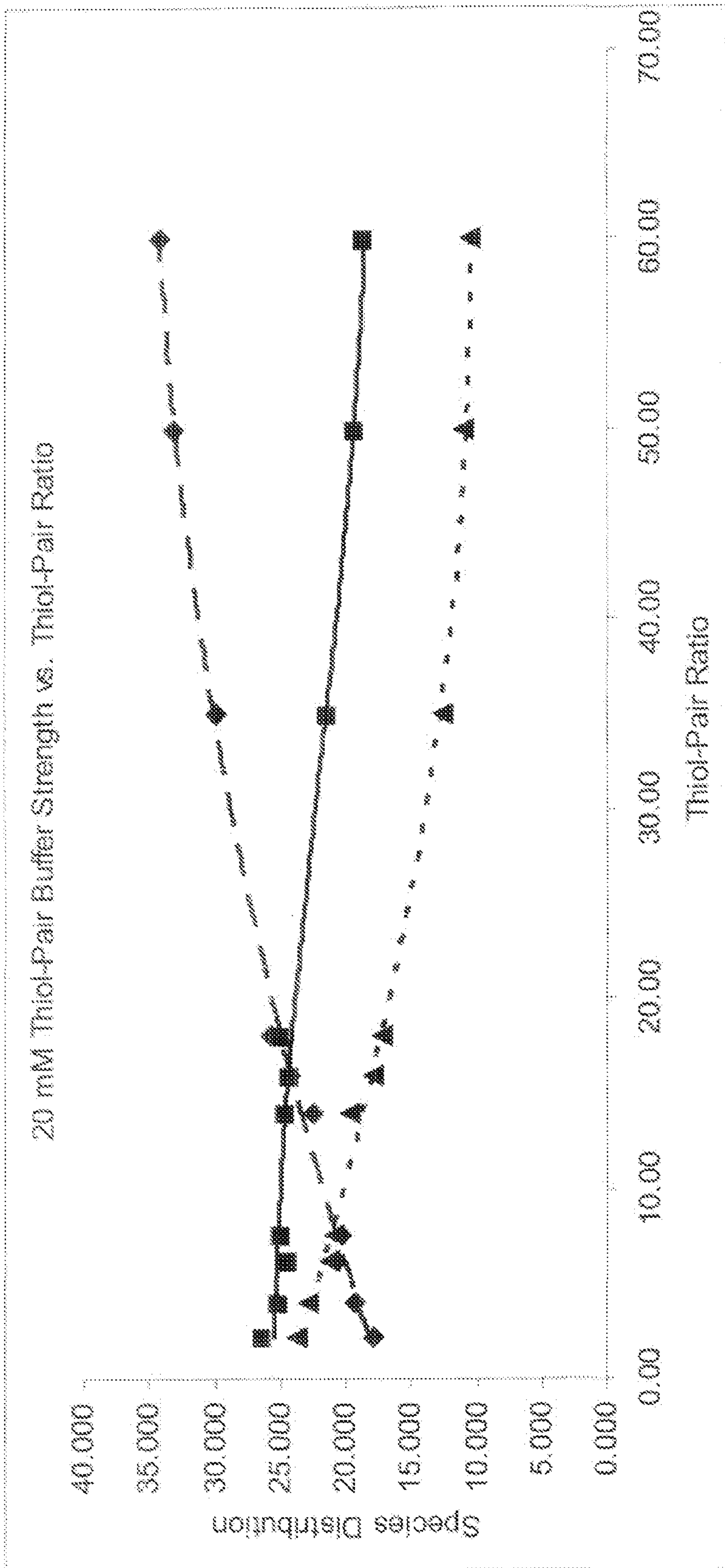
20 mM Thiol-Pair Buffer Strength vs. Thiol-Pair Ratio
Species Distribution
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
Thiol-Pair Ratio

Figure 1f

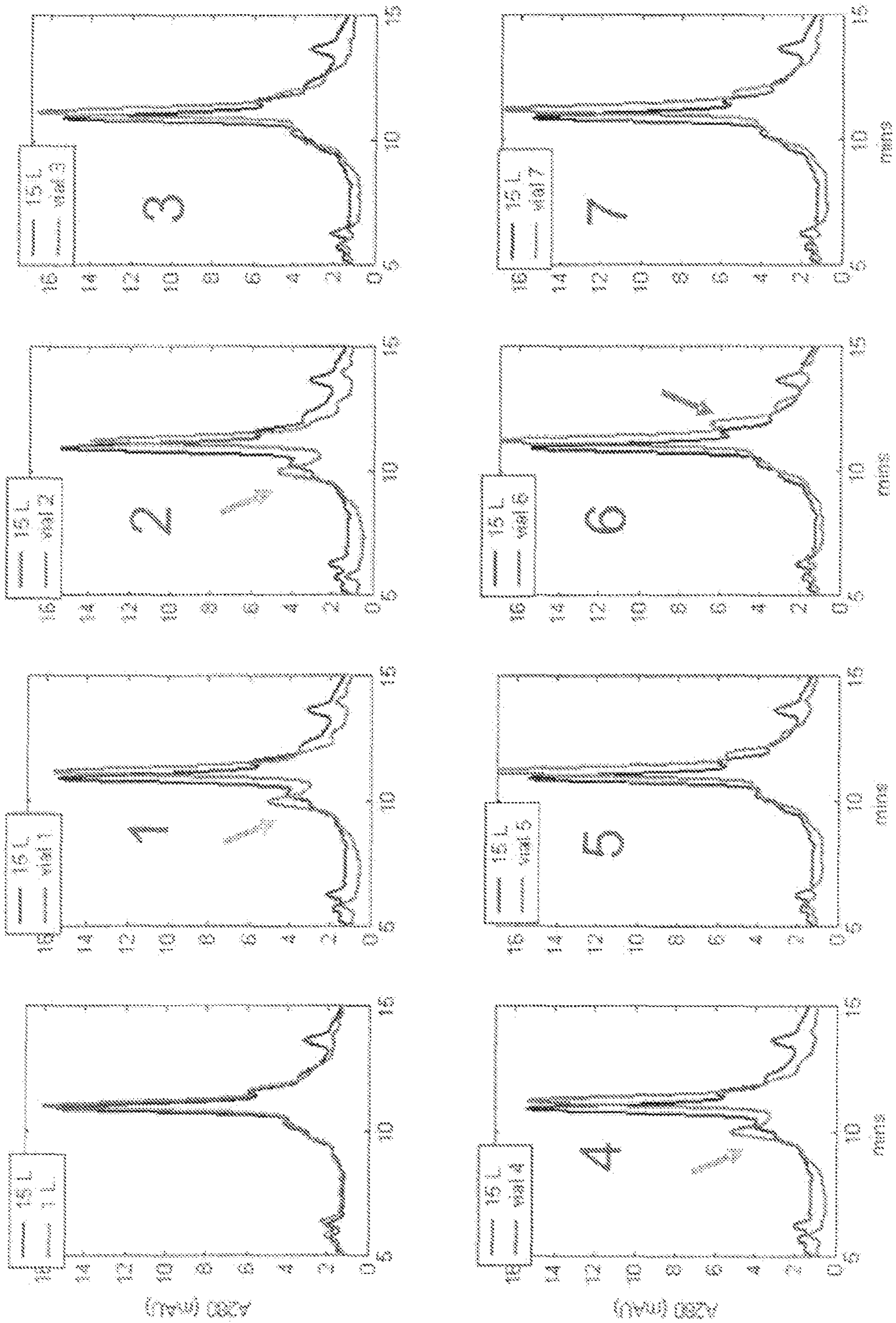
15 L
1 L
15 L
vial 1
15 L
vial 2
15 L
vial 3
15 L
vial 4
15 L
vial 5
15 L
vial 6
15 L
vial 7
1
2
3
4
5
6
7
A280 (mAU)
mins

Figure 2

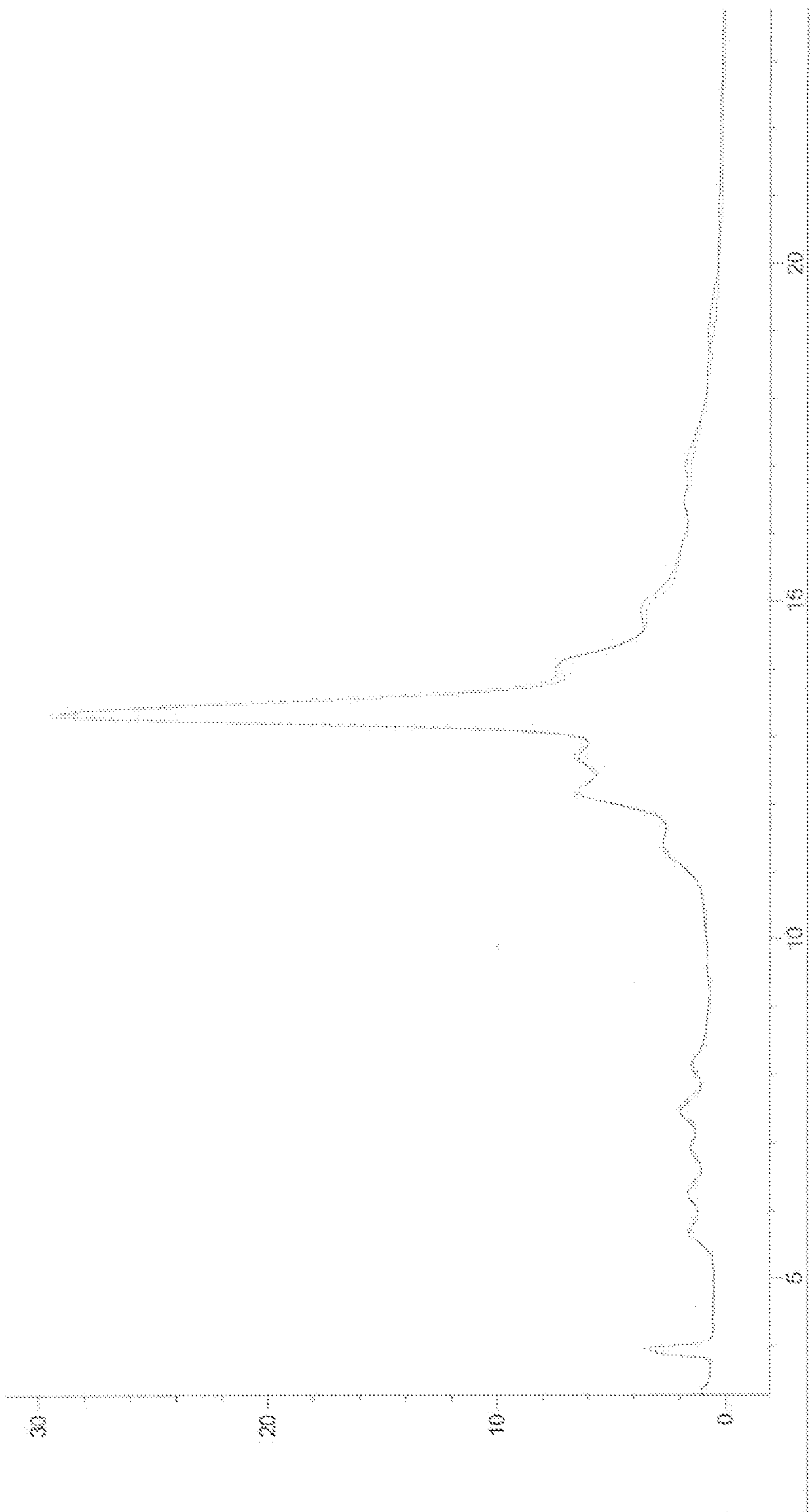

Figure 3

REFOLDING PROTEINS USING A CHEMICALLY CONTROLLED REDOX STATE

This application claims the benefit of U.S. Provisional Application No. 61/219,257 filed Jun. 22, 2009, which is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention generally relates to refolding proteins at high concentrations, and more particularly to refolding proteins in volumes at concentrations of 2.0 g/L and above.

BACKGROUND OF THE INVENTION

Recombinant proteins can be expressed in a variety of expression systems, including non-mammalian cells, such as bacteria and yeast. A difficulty associated with the expression of recombinant proteins in prokaryotic cells, such as bacteria, is the precipitation of the expressed proteins in limited-solubility intracellular precipitates typically referred to as inclusion bodies. Inclusion bodies are formed as a result of the inability of a bacterial host cell to fold recombinant proteins properly at high levels of expression and as a consequence the proteins become insoluble. This is particularly true of prokaryotic expression of large, complex or protein sequences of eukaryotic origin. Formation of incorrectly folded recombinant proteins has, to an extent, limited the commercial utility of bacterial fermentation to produce recombinant large, complex proteins, at high levels of efficiency.

Since the advent of the recombinant expression of proteins at commercially viable levels in non-mammalian expression systems such as bacteria, various methods have been developed for obtaining correctly folded proteins from bacterial inclusion bodies. These methods generally follow the procedure of expressing the protein, which typically precipitates in inclusion bodies, lysing the cells, collecting the inclusion bodies and then solubilizing the inclusion bodies in a solubilization buffer comprising a denaturant or surfactant and optionally a reductant, which unfolds the proteins and disassembles the inclusion bodies into individual protein chains with little to no structure. Subsequently, the protein chains are diluted into or washed with a refolding buffer that supports renaturation to a biologically active form. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (e.g., a redox system).

Typical refold concentrations for complex molecules, such as molecules comprising two or more disulfides, are less than 2.0 g/L and more typically 0.01-0.5 g/L (Rudolph & Lilie, (1996) *FASEB J.* 10:49-56). Thus, refolding large masses of a complex protein, such as an antibody, peptibody or other Fc fusion protein, at industrial production scales poses significant limitations due to the large volumes required to refold proteins, at these typical product concentration, and is a common problem facing the industry. One factor that limits the refold concentration of these types of proteins is the formation of incorrectly paired disulfide bonds, which may in turn increase the propensity for those forms of the protein to aggregate. Due to the large volumes of material and large pool sizes involved when working with industrial scale protein production, significant time, and resources can be saved by eliminating or simplifying one or more steps in the process.

While protein refolding has previously been demonstrated at higher concentrations, the proteins that were refolded were either significantly smaller in molecular weight, less complex molecules containing only one or two disulfide bonds (see, e.g., Creighton, (1974) *J. Mol. Biol.* 87:563-577). Additionally, the refolding processes for such proteins employed detergent-based refolding chemistries (see, e.g., Stockel et al., (1997) *Eur J Biochem* 248:684-691) or utilized high pressure folding strategies (St John et al., (2001) *J. Biol. Chem.* 276(50):46856-63). More complex molecules, such as antibodies, peptibodies and other large proteins, are generally not amenable to detergent refold conditions and are typically refolded in chaotropic refold solutions. These more complex molecules often have greater than two disulfide bonds, often between 8 and 24 disulfide bonds, and can be multi-chain proteins that form homo- or hetero-dimers.

Until the present disclosure, these types of complex molecules could not be refolded at high concentrations, i.e., concentrations of 2.0 g/L and higher, with any meaningful degree of efficiency on a small scale, and notably not on an industrial scale. The disclosed methods, in contrast, can be performed at high concentrations on a small or large (e.g, industrial) scale to provide properly refolded complex proteins. The ability to refold proteins at high concentrations and at large scales can translate into not only enhanced efficiency of the refold operation itself, but also represents time and cost savings by eliminating the need for additional equipment and personnel. Accordingly, a method of refolding proteins present in high concentrations could translate into higher efficiencies and cost savings to a protein production process.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a series of plots depicting depicting the effect of thiol-pair ratio and redox buffer strength on product-species distribution; FIG. **1***a* depicts the effect of a 5 mM buffer strength; FIG. **1***b* depicts the effect of a 7.5 mM buffer strength; FIG. **1***c* depicts the effect of a 10 mM buffer strength; FIG. **1***d* depicts the effect of a 12.5 mM buffer strength; FIG. **1***e* depicts the effect of a 15 mM buffer strength and FIG. **1***f* depicts the effect of a 20 mM buffer strength.

FIG. **2** is a series of plots depicting the effect of the degree of aeration on the species distribution under fixed thiol-pair ratio and thiol-pair buffer strength.

FIG. **3** is an analytical overlay of a chemically controlled, non-aerobic refold performed at 6 g/L and optimized using an embodiment of the described method performed at 1 L and 2000 L.

SUMMARY OF THE INVENTION

A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising: (a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater and one or more of: (i) a denaturant; (ii) an aggregation suppressor; and (iii) a protein stabilizer; to form a refold mixture; (b) incubating the refold mixture; and (c) isolating the protein from the refold mixture.

In various embodiments the redox component has a final thiol-pair ratio greater than or equal to 0.001 but less than or equal to 100, for example within a range of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50 and a Thiol-pair buffer strength equal to or greater than 2 mM, for

example greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM. Restated, in terms of ranges, the thiol buffer strength can be between 2 and 20 mM, for example between 2.25 mM and 20 mM, 2.5 mM and 20 mM, 2.75 mM and 20 mM, 3 mM and 20 mM, 5 mM and 20 mM, 7.5 mM and 20 mM, 10 mM and 20 mM, or 15 mM and 20 mM, to form a mixture.

In one embodiment of a refold buffer, the refold buffer comprises urea, arginine-HCl, cysteine and cystamine in Tris buffer. In a further embodiment the components are present in the refold buffer in proportions described in Example 3.

In another embodiment of a refold buffer, the refold buffer comprises urea, arginine HCl, glycerol, cysteine, and cystamine in Tris buffer. In a further embodiment the components are present in the refold buffer in proportions described in Example 4.

In some embodiments, the protein is initially present in a volume in a non-native limited solubility form, such as an inclusion body. Alternatively, the protein is present in the volume in a soluble form. The protein can be a recombinant protein or it can be an endogenous protein. The protein can be a complex protein such as an antibody or a multimeric protein. In another embodiment, the protein is an Fc-protein conjugate, such as a protein fused or linked to a Fc domain.

The non-mammalian expression system can be a bacterial expression system or a yeast expression system.

The denaturant in the refold buffer can be selected from the group consisting of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea. The protein stabilizer in the refold buffer can be selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes. The aggregation suppressor can be selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes. The thiol-pairs can comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

In various embodiments, the purification can comprise contacting the mixture with an affinity separation matrix, such as a Protein A or Protein G resin. Alternatively, the affinity resin can be a mixed mode separation matrix or an ion exchange separation matrix. In various aspects, the incubation can be performed under aerobic conditions or under non-aerobic conditions.

DETAILED DESCRIPTION OF THE INVENTION

The relevant literature suggests that when optimizing various protein refolding operations, the refold buffer thiol-pair ratio has been purposefully varied and as a result the thiol buffer strength was unknowingly varied across a wide range of strengths (see, e.g., Lille, Schwarz & Rudolph, (1998) *Current Opinion in Biotechnology* 9(5):497-501, and Tran-Moseman, Schauer & Clark (1999) *Protein Expression & Purification* 16(1):181-189). In one study, a relationship between the thiol pair ratio and the buffer strength was investigated for lysozyme, a simple, single-chain protein that forms a molten globule. (De Bernardez et al., (1998) *Biotechnol. Prog.* 14:47-54). The De Bernardez work described thiol concentration in terms of a model that considered only the kinetics of a one-way reaction model. However, most complex proteins are governed by reversible thermodynamic equilibria that are not as easily described (see, e.g., Darby et al., (1995) *J. Mol. Biol.* 249:463-477). More complex behavior is expected in the case of large multi-chain proteins containing many disulfide bonds, such as antibodies, peptibodies and other Fc fusion proteins. Until the present disclosure, specific relationships had not been provided for thiol buffer strength, thiol-pair ratio chemistry, and protein concentration with respect to complex proteins that related to the efficiency of protein production. Consequently, the ability to refold proteins in a highly concentrated volume has largely been an inefficient or unachievable goal, leading to bottlenecks in protein production, particularly on the industrial scale.

Prior to the present disclosure a specific controlled investigation of the independent effects of thiol-pair ratio and thiol-pair buffer strength had not been disclosed for complex proteins. As described herein, by controlling the thiol-pair buffer strength, in conjunction with thiol-pair ratio and protein concentration, the efficiency of protein folding operations can be optimized and enhanced and the refolding of proteins at high concentrations, for example 2 g/L or greater, can be achieved.

Thus, in one aspect, the present disclosure relates to the identification and control of redox thiol-pair ratio chemistries that facilitate protein refolding at high protein concentrations, such as concentrations higher than 2.0 g/L. The method can be applied to any type of protein, including simple proteins and complex proteins (e.g., proteins comprising 2-23 disulfide bonds or greater than 250 amino acid residues, or having a MW of greater than 20,000 daltons), including proteins comprising a Fc domain, such as antibodies, peptibodies and other Fc fusion proteins, and can be performed on a laboratory scale (typically milliliter or liter scale), a pilot plant scale (typically hundreds of liters) or an industrial scale (typically thousands of liters). Examples of complex molecules known as peptibodies, and other Fc fusions, are described in U.S. Pat. Nos. 6,660,843, 7,138,370 and 7,511,012.

As described herein, the relationship between thiol buffer strength and redox thiol-pair ratio has been investigated and optimized in order to provide a reproducible method of refolding proteins at concentrations of 2.0 g/L and higher on a variety of scales. A mathematical formula was deduced to allow the precise calculation of the ratios and strengths of individual redox couple components to achieve matrices of buffer thiol-pair ratio and buffer thiol strength. Once this relationship was established, it was possible to systematically demonstrate that thiol buffer strength and the thiol-pair ratio interact to define the distribution of resulting product-related species in a refolding reaction.

The buffer thiol-pair ratio is, however, only one component in determining the total system thiol-pair ratio in the total reaction. Since the cysteine residues in the unfolded protein are reactants as well, the buffer thiol strength needs to vary in proportion with increases in protein concentration to achieve the optimal system thiol-pair ratio. Thus, in addition to demonstrating that buffer thiol strength interacts with the thiol-pair ratio, it has also been shown that the buffer thiol strength relates to the protein concentration in the total reaction as well. Optimization of the buffer thiol strength and the system thiol pair ratio can be tailored to a particular protein, such as a complex protein, to minimize cysteine mispairing yet still facilitate a refold at a high concentration.

I. Definitions

As used herein, the terms "a" and "an" mean one or more unless specifically indicated otherwise.

As used herein, the term "non-mammalian expression system" means a system for expressing proteins in cells derived from an organism other than a mammal, including but not limited to, prokaryotes, including bacteria such as *E. coli*, and yeast. Often a non-mammalian expression system is

employed to express a recombinant protein of interest, while in other instances a protein of interest is an endogenous protein that is expressed by a non-mammalian cell. For purposes of the present disclosure, regardless of whether a protein of interest is endogenous or recombinant, if the protein is expressed in a non-mammalian cell then that cell is a "non-mammalian expression system." Similarly, a "non-mammalian cell" is a cell derived from an organism other than a mammal, examples of which include bacteria or yeast.

As used herein, the term "denaturant" means any compound having the ability to remove some or all of a protein's secondary and tertiary structure when placed in contact with the protein. The term denaturant refers to particular chemical compounds that affect denaturation, as well as solutions comprising a particular compound that affect denaturation. Examples of denaturants that can be employed in the disclosed method include, but are not limited to urea, guanidinium salts, dimethyl urea, methylurea, ethylurea and combinations thereof.

As used herein, the term "aggregation suppressor" means any compound having the ability to disrupt and decrease or eliminate interactions between two or more proteins. Examples of aggregation suppressors can include, but are not limited to, amino acids such as arginine, proline, and glycine; polyols and sugars such as glycerol, sorbitol, sucrose, and trehalose; surfactants such as, polysorbate-20, CHAPS, Triton X-100, and dodecyl maltoside; and combinations thereof.

As used herein, the term "protein stabilizer" means any compound having the ability to change a protein's reaction equilibrium state, such that the native state of the protein is improved or favored. Examples of protein stabilizers can include, but are not limited to, sugars and polyhedric alcohols such as glycerol or sorbitol; polymers such as polyethylene glycol (PEG) and α-cyclodextrin; amino acids salts such as arginine, proline, and glycine; osmolytes and certain Hoffmeister salts such as Tris, sodium sulfate and potassium sulfate; and combinations thereof.

As used herein, the terms "Fc" and "Fc region" are used interchangeably and mean a fragment of an antibody that comprises human or non-human (e.g., murine) C_{H2} and C_{H3} immunoglobulin domains, or which comprises two contiguous regions which are at least 90% identical to human or non-human C_{H2} and C_{H3} immunoglobulin domains. An Fc can but need not have the ability to interact with an Fc receptor. See, e.g., Hasemann & Capra, "Immunoglobulins: Structure and Function," in William E. Paul, ed., *Fundamental Immunology*, Second Edition, 209, 210-218 (1989), which is incorporated by reference herein in its entirety.

As used herein, the terms "protein" and "polypeptide" are used interchangeably and mean any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds.

As used herein, the terms "isolated" and "purify" are used interchangeably and mean to reduce by 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95%, or more, the amount of heterogenous elements, for example biological macromolecules such as proteins or DNA, that may be present in a sample comprising a protein of interest. The presence of heterogenous proteins can be assayed by any appropriate method including High-performance Liquid Chromatography (HPLC), gel electrophoresis and staining and/or ELISA assay. The presence of DNA and other nucleic acids can be assayed by any appropriate method including gel electrophoresis and staining and/or assays employing polymerase chain reaction.

As used herein, the term "complex molecule" means any protein that is (a) larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form. A complex molecule can, but need not, form multimers. Examples of complex molecules include but are not limited to, antibodies, peptibodies and other chimeric molecules comprising an Fc domain and other large proteins. Examples of complex molecules known as peptibodies, and other Fc fusions, are described in U.S. Pat. Nos. 6,660,843, 7,138,370 and 7,511,012.

As used herein, the term "peptibody" refers to a polypeptide comprising one or more bioactive peptides joined together, optionally via linkers, with an Fc domain. See U.S. Pat. Nos. 6,660,843, 7,138,370 and 7,511,012 for examples of peptibodies.

As used herein, the term "refolding" means a process of reintroducing secondary and tertiary structure to a protein that has had some or all of its native secondary or tertiary structure removed, either in vitro or in vivo, e.g., as a result of expression conditions or intentional denaturation and/or reduction. Thus, a refolded protein is a protein that has had some or all of its native secondary or tertiary structure reintroduced.

As used herein, the term "buffer thiol-pair ratio" is defined by the relationship of the reduced and oxidized redox species used in the refold buffer as defined in Equation 1:

Definition of Buffer Thiol-Pair Ratio (TPR) Equation 1

$$\text{Buffer } TPR = \frac{[\text{reductant}]^2}{[\text{oxidant}]} = \frac{[\text{cysteine}]^2}{[\text{cystamine}]}.$$

As used herein, the terms "Buffer Thiol Strength", "Thiol-Pair Buffer Strength", and "Thiol-pair Strength" are used interchangeably and are defined in Equation 2, namely as the total mono-equivalent thiol concentration, wherein the total concentration is the sum of the reduced species and twice the concentration of the oxidized species.

Definition of Buffer Thiol-Pair Buffer Strength/Thiol Buffer Strength (BS) Thiol-Pair Buffer Strength=2[oxidant]+[reductant]=2[cystamine]+[cysteine] Equation 2.

The relationship between the thiol-pair ratio and thiol-pair buffer strength is described in equations 3 and 4.

Calculation of the Reduced Redox Species with Regard to a Defined Redox Buffer Strength (BS) and buffer Redox Potential Equation 3

$$\text{Concentration of Reduced Redox Component} = \frac{\left(\sqrt{bufferTPR^2 + 8 * bufferTPR * BS}\right) - bufferTPR}{4}.$$

Calculation of the Oxidized Redox Species with Regard to a Defined Redox Buffer Strength (BS) and Buffer Redox Potential Equation 4

$$\text{Concentration of Oxidized Redox Component} = \frac{(\text{Concentration of Reduced Redox Component})^2}{TPR}.$$

As used herein, the term "redox component" means any thiol-reactive chemical or solution comprising such a chemical that facilitates a reversible thiol exchange with another thiol or the cysteine residues of a protein. Examples of such compounds include, but are not limited to, glutathione-re-

duced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine, beta-mercaptoethanol and combinations thereof.

As used herein, the term "solubilization" means a process in which salts, ions, denaturants, detergents, reductants and/or other organic molecules are added to a solution comprising a protein of interest, thereby removing some or all of a protein's secondary and/or tertiary structure and dissolving the protein into the solvent. This process can include the use of elevated temperatures, typically 10-50° C., but more typically 15-25° C., and/or alkaline pH, such as pH 7-12. Solubilization can also be accomplished by the addition of acids, such as 70% formic acid (see, e.g., Cowley & Mackin (1997) *FEBS Lett* 402:124-130).

A "solubilized protein" is a protein in which some or all of the protein's secondary and/or tertiary structure has been removed.

A "solublization pool" is a volume of solution comprising a solubilized protein of interest as well as the salts, ions, denaturants, detergents, reductants and/or other organic molecules selected to solubilize the protein.

As used herein, the term "non-aerobic condition" means any reaction or incubation condition that is performed without the intentional aeration of the mixture by mechanical or chemical means. Under non-aerobic conditions oxygen can be present, as long as it is naturally present and was not introduced into the system with the intention of adding oxygen to the system. Non-aerobic conditions can be achieved by, for example, limiting oxygen transfer to a reaction solution by limiting headspace pressure, the absence of, or limited exposure to, air or oxygen contained in the holding vessel, air or oxygen overlay, the lack of special accommodations to account for mass transfer during process scaling, or the absence of gas sparging or mixing to encourage the presence of oxygen in the reaction system. Non-aerobic conditions can also be achieved by intentionally limiting or removing oxygen from the system via chemical treatment, headspace overlays or pressurization with inert gases or vacuums, or by sparging with gases such as argon or nitrogen, results in the reduction of oxygen concentration in the reaction mixture.

As used herein, the terms "non-native" and "non-native form" are used interchangeably and when used in the context of a protein of interest, such as a protein comprising a Fc domain, mean that the protein lacks at least one formed structure attribute found in a form of the protein that is biologically active in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity. Examples of structural features that can be lacking in a non-native form of a protein can include, but are not limited to, a disulfide bond, quaternary structure, disrupted secondary or tertiary structure or a state that makes the protein biologically inactive in an appropriate assay. A protein in a non-native form can but need not form aggregates.

As used herein, the term "non-native limited solubility form" when used in the context of a protein of interest, such as a protein comprising a Fc domain, means any form or state in which the protein lacks at least one formed structural feature found in a form of the protein that (a) is biologically active in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity and/or (b) forms aggregates that require treatment, such as chemical treatment, to become soluble. The term specifically includes proteins existing in inclusion bodies, such as those sometimes found when a recombinant protein is expressed in a non-mammalian expression system.

II. Theory

Refolding microbial-derived molecules present in a pool at concentrations of 2.0 g/L or higher is advantageous for a variety of reasons, primarily because of the associated reduction in reaction volumes and increases in process throughput. From a process scaling standpoint, it is advantageous to refold under conditions that do not require aerobic conditions; such conditions can be achieved, for example, by constant or intermittent sparging, the implementation of air or oxygen headspace overlays, by pressurizing the headspace, or by employing high efficiency mixing. Since the oxygen concentration in the system is related to mass transfer, the scaling of the refold reaction becomes considerably more difficult as factors such as tank geometry, volume, and mixing change. Furthermore, oxygen may not be a direct reactant in the formation of disulfide bonds in the protein, making a direct link to the mass transfer coefficient unlikely. This further complicates scaling of the reaction. Therefore, non-aerobic, chemically controlled redox systems are preferred for refolding proteins. Examples of such conditions are provided herein.

The optimal refold chemistry for a given protein represents a careful balance that maximizes the folded/oxidized state while minimizing undesirable product species, such as aggregates, unformed disulfide bridges (e.g., reduced cysteine pairs), incorrect disulfide pairings (which can lead to misfolds), oxidized amino acid residues, deamidated amino acid residues, incorrect secondary structure, and product-related adducts (e.g., cysteine or cysteamine adducts). One factor that is important in achieving this balance is the redox-state of the refold system. The redox-state is affected by many factors, including, but not limited to, the number of cysteine residues contained in the protein, the ratio and concentration of the redox couple chemicals in the refold solution (e.g., cysteine, cystine, cystamine, cysteamine, glutathione-reduced and glutathione-oxidized), the concentration of reductant carried over from the solubilization buffer (e.g., DTT, glutathione and beta-mercaptoethanol), the level of heavy metals in the mixture, and the concentration of oxygen in the solution.

Thiol-pair ratio and thiol-pair buffer strength are defined in Equations 1 and 2, infra, using cysteine and cystamine as an example reductant and oxidant, respectively. These quantities, coupled with protein concentration and reductant carryover from the solubilization, can be factors in achieving a balance between the thiol-pair ratio and the thiol-pair buffer strength.

Turning to FIG. **1**, this figure depicts the effect of thiol-pair ratio and thiol buffer strength on the distribution of product-related species, as visualized by reversed phase-HPLC analysis, for a complex dimeric protein. In FIGS. **1***a*-**1***f*, the dotted lines represent protein species with oxidized amino acid residues, single chain species, and stable mixed disulfide intermediates, the dashed lines represent mis-paired or incorrectly formed disulfide protein species and protein species with partially unformed disulfide linkages. The solid lines represent properly folded protein species. FIGS. **1***a*-**1***f* demonstrate that at a constant 6 g/L protein concentration, as the thiol-pair buffer strength is increased, the thiol-pair ratio required to achieve a comparable species distribution must also increase. For example, as shown in FIG. **1**, if the buffer strength is increased to 10 mM, from 5 mM, the balanced thiol-pair ratio would be about 2-fold higher, to achieve a comparable species distribution. This is largely due to increased buffering of the reductant carried over from the solubilization, on the total system thiol-pair ratio. At lower redox buffer strengths, the overall system becomes much more difficult to control. The protein concentration and number of cysteines contained in the protein sequence also relate to the minimum required thiol-pair buffer strength required to control the system. Below a certain point, which will vary from protein to protein,

the protein thiol concentration can overwhelm the redox couple chemistry and lead to irreproducible results.

In the results depicted in FIG. 1, when the thiol-pair ratio of the refolding solution is intentionally set to be more reducing, the resultant product distribution shifts to produce more of the reduced product species (dashed lines). When the Thiol-Pair Ratio of the refolding solution is intentionally set to be lower, or more oxidizing, the resultant product distribution shifts to produce more oxidized residues, single chain forms, and stable mixed disulfide intermediate species (dotted lines). The ability to select an optimal Thiol-Pair Ratio and Thiol-pair Buffer Strength allows for the optimization of the yield of a desired folded protein form. This optimized yield can be achieved by maximizing the mass or yield of desired folded protein species in the refolding pool or by purposefully shifting the resultant undesired product-related species to a form that is most readily removed in the subsequent purification steps and thusly leads to an overall benefit to process yield or purity.

Optimization of the redox component Thiol-pair Ratios and Thiol-pair Buffer Strengths can be performed for each protein. A matrix or series of multifactorial matrices can be evaluated to optimize the refolding reaction for conditions that optimize yield and distributions of desired species. An optimization screen can be set up to systematically evaluate redox chemistries, Thiol-pair ratios, Thiol-pair Buffer Strengths, incubation times, protein concentration and pH in a full or partial factorial matrix, with each component varied over a range of at least three concentration or pH levels with all other parameters kept constant. The completed reactions can be evaluated by RP-HPLC and SE-HPLC analysis for yield and product quality using standard multivariate statistical tools.

III. Method Of Refolding A Protein Expressed In A Non-Mammalian Expression System And Present In A Volume At A Concentration Of 2.0 G/L Or Greater

The disclosed refold method is particularly useful for refolding proteins expressed in non-mammalian expression systems. As noted herein, non-mammalian cells can be engineered to produce recombinant proteins that are expressed intracellularly in either a soluble or a completely insoluble or non-native limited solubility form. Often the cells will deposit the recombinant proteins into large insoluble or limited solubility aggregates called inclusion bodies. However, certain cell growth conditions (e.g., temperature or pH) can be modified to drive the cells to produce a recombinant protein in the form of intracellular, soluble monomers. As an alternative to producing proteins in insoluble inclusion bodies, proteins can be expressed as soluble proteins, including proteins comprising an Fc region, which can be captured directly from cell lysate by affinity chromatography. Capturing directly from lysate allows for the refolding of relatively pure protein and avoids the very intensive harvesting and separation process that is required in inclusion body processes. The refolding method, however, is not limited to samples that have been affinity purified and can be applied to any sample comprising a protein that was expressed in a non-mammalian expression system, such as a protein found in a volume of cell lysate (i.e., a protein that has not been purified in any way).

In one aspect, the present disclosure relates to a method of refolding a protein expressed in a non-mammalian expression system in a soluble form and present in a volume at a concentration of 2.0 g/L or greater, such as a protein that has been purified by affinity chromatography from the cell lysate of

non-mammalian cells in which the protein was expressed. Although the volume can be derived from any stage of a protein purification process, in one example the volume is an affinity chromatography elution pool (e.g., a Protein A elution pool). In another example, the volume is situated in a process stream. The method is not confined to Fc-containing proteins, however, and can be applied to any kind of peptide or protein that is expressed in a soluble form and captured from non-mammalian-derived cell lysate. The isolated soluble protein is often released from non-mammalian cells in a reduced form and therefore can be prepared for refolding by addition of a denaturant, such as a chaotrope. Further combination with protein stabilizers, aggregation suppressors and redox components, at an optimized Thiol-pair ration and Thiol-pair Buffer Strength, allows for refolding at concentrations of 1-40 g/L, for example at concentrations of 10-20 g/L.

In one particular embodiment of the method, a protein is expressed in a non-mammalian expression system, and is released from the expressing cell by high pressure lysis. The protein is then captured from the lysate by Protein A affinity chromatography and is present in a volume at a concentration of 10 g/L or greater. The protein is then contacted with a refold buffer comprising a denaturant, an aggregation suppressor, a protein stabilizer and a redox component, wherein the redox component has a final thiol-pair ratio (as defined herein) having a range of 0.001 to 100, for example within a range of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50 and a Thiol-pair buffer strength (as defined herein) equal to or greater than 2 mM, for example greater than or equal to 2.25 mM, 2.5, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM. Restated, in terms of ranges, the thiol buffer strength is between 2 and 20 mM, for example between 2.25 mM and 20 mM, 2.5 mM and 20 mM, 2.75 mM and 20 mM, 3 mM and 20 mM, 5 mM and 20 mM, 7.5 mM and 20 mM, 10 mM and 20 mM, or 15 mM and 20 mM.

In another aspect, the present disclosure relates to a method of refolding a protein expressed in a non-mammalian expression system in an insoluble or limited-solubility form, such as in the form of inclusion bodies. When the protein is disposed in inclusion bodies, the inclusion bodies can be harvested from lysed cells, washed, concentrated and refolded.

Optimization of the refold buffer can be performed for each protein and each final protein concentration level using the novel method provided herein. As shown in the Examples, good results can be obtained when refolding a protein comprising an Fc region when the refold buffer contains a denaturant (e.g., urea or other chaotrope, organic solvent or strong detergent), aggregation suppressors (e.g., a mild detergent, arginine or low concentrations of PEG), protein stabilizers (e.g., glycerol, sucrose or other osmolyte, salts) and redox components (e.g., cysteine, cystamine, glutathione). The optimal thiol-pair ratio and redox buffer strength can be determined using an experimental matrix of thiol-pair ratio (which can have a range of 0.001 to 100, for example within a range of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50) versus thiol-pair buffer strength (which can be greater than 2 mM, for example greater than or equal to 2.25 mM, 2.5, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM. Restated, in terms of ranges, the thiol buffer strength is between 2 and 20 mM, for example between 2.25 mM and 20 mM, 2.5 mM and 20 mM, 2.75 mM and 20 mM, 3 mM and 20 mM, 5 mM and 20 mM,

7.5 mM and 20 mM, 10 mM and 20 mM, or 15 mM and 20 mM, depending on the protein concentration and the concentration of reductant used to solubilize the inclusion bodies). Conditions can be optimized using the novel methods described in Example 2.

In one particular embodiment of the method, a protein is expressed in a non-mammalian expression system and is present in a volume at a concentration of 2.0 g/L or greater. The protein is contacted with a refold buffer comprising a denaturant, an aggregation suppressor, a protein stabilizer and a redox component, wherein the redox component has a final thiol-pair ratio (as defined herein) having a range of 0.001 to 100, for example within a range of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50, and a Thiol-pair buffer strength (as defined herein) equal to or greater than 2 mM, for example greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM. Restated, in terms of ranges, the thiol buffer strength is between 2 and 20 mM, for example between 2.25 mM and 20 mM, 2.5 mM and 20 mM, 2.75 mM and 20 mM, 3 mM and 20 mM, 5 mM and 20 mM, 7.5 mM and 20 mM, 10 mM and 20 mM, or 15 mM and 20 mM.to form a mixture. A wide range of denaturant types may be employed in the refold buffer. Examples of some common denaturants that can be employed in the refold buffer include urea, guanidinium, dimethyl urea, methylurea, or ethylurea. The specific concentration of the denaturant can be determined by routine optimization, as described herein.

A wide range of protein stabilizers or aggregation suppressors can be employed in the refold buffer. Examples of some common aggregation suppressors that can be useful in the refold buffer include arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate, other osmolytes, or similar compounds. The specific concentration of the aggregation suppressor can be determined by routine optimization, as described herein.

A redox component of the refold buffer can be of any composition, with the caveat that the redox component has a final thiol-pair ratio in a range of 0.001 to 100, for example within a range of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 or 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50, and a Thiol-pair buffer strength of greater than or equal to 2 mM, for example greater than or equal to 2.25 mM, 2.5, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM, or 15 mM, wherein the thiol-pair buffer strength is effectively bounded at a maximum of 100 mM. Restated, in terms of ranges, the thiol buffer strength is between 2 and 20 mM, for example between 2.25 mM and 20 mM, 2.5 mM and 20 mM, 2.75 mM and 20 mM, 3 mM and 20 mM, 5 mM and 20 mM, 7.5 mM and 20 mM, 10 mM and 20 mM, or 15 mM and 20 mM. Methods of identifying a suitable redox component, i.e., determining appropriate thiol-pair ratios and redox buffer strengths, are known and/or are provided herein. Examples of specific thiol pairs that can form the redox component can include one or more of reduced glutathione, oxidized glutathione, cysteine, cystine, cysteamine, cystamine, and beta-mercaptoethanol. Thus, a thiol-pair can comprise, for example, reduced glutathione and oxidized glutathione. Another example of a thiol pair is cysteine and cystamine. The redox component can be optimized as described herein.

After the protein has been contacted with a redox component having the recited thiol pair ratio and redox buffer strength to form a refold mixture, the refold mixture is then incubated for a desired period of time. The incubation can be

performed under non-aerobic conditions, as defined herein. Non-aerobic conditions need not be completely free of oxygen, only that no additional oxygen other than that present in the initial system is purposefully introduced. The incubation period is variable and is selected such that a stable refold mixture can be achieved with the desired analytical properties. An incubation period can be, for example, 1 hour, 4 hours, 12 hours, 24 hours, 48 hours, 72 hours, or longer.

Due to the sensitivity of high concentration refolds to the level of oxygen present in the system and the tendency for oxygen mass transfer to be greater at small-scale, a methodology and/or apparatus can be developed to control the oxygen levels and maintain non-aerobic conditions for the incubation step. In one embodiment, the procedure can comprise the preparation, dispensing and mixing of all refold components under a blanket of inert gas, such as nitrogen or argon, to avoid entraining levels of oxygen into the reaction. This approach is particularly helpful in identifying an acceptable thiol-pair ratio. In another embodiment useful at scales of 15 liters or less, the headspace of the refold reactor containing the protein and refold buffer can be purged with an inert gas or a mixture of inert gas and air or oxygen, and the reaction vessel sealed and mixed at a low rotational speed for the duration of the incubation time.

Following the incubation, the protein is isolated from the refold mixture. The isolation can be achieved using any known protein purification method. If the protein comprises a Fc domain, for example, a Protein A column provides an appropriate method of separation of the protein from the refold excipients. In other embodiments, various column chromatography strategies can be employed and will depend on the nature of the protein being isolated. Examples include HIC, AEX, CEX and SEC chromatography. Non-chromatographic separations can also be considered, such as precipitation with a salt, acid or with a polymer such as PEG (see, e.g., US 20080214795). Another alternative method for isolating the protein from the refold components can include dialysis or diafiltration with a tangential-flow filtration system.

In another exemplary refolding operation, inclusion bodies obtained from a non-mammalian expression system are solubilized in the range of 10 to 100 grams of protein per liter and more typically from 20-40 g/L for approximately 10-300 min. The solubilized inclusion bodies are then diluted to achieve reduction of the denaturants and reductants in the solution to a level that allows the protein to refold. The dilution results in protein concentration in the range of 1 to 15 g/L in a refold buffer containing urea, glycerol or sucrose, arginine, and the redox pair (e.g., cysteine and cystamine). In one embodiment the final composition is 1-4 M urea, 5-40% glycerol or sucrose, 25-500 mM arginine, 0.1-10 mM cysteine and 0.1-10 mM cystamine. The solution is then mixed during incubation over a time that can span from 1 hour to 4 days.

As noted herein, the disclosed method is particularly useful for proteins expressed in bacterial expression systems, and more particularly in bacterial systems in which the protein is expressed in the form of inclusion bodies within the bacterial cell. The protein can be a complex protein, i.e., a protein that (a) is larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form. When the protein is expressed in an inclusion body it is likely that any disulfide bond found in the protein's native form will be misformed or not formed at all. The disclosed method is applicable to these and other forms of a protein of interest. Specific examples of proteins that can be considered for refolding using the disclosed methods include antibodies, which are traditionally very difficult to

refold at high concentrations using typical refold methods due to their relatively large size and number of disulfide bonds. The method can also be employed to refold other Fc-containing molecules such as peptibodies, and more generally to refold any fusion protein comprising an Fc domain fused to another protein.

Another aspect of the disclosed method is its scalability, which allows the method to be practiced on any scale, from bench scale to industrial or commercial scale. Indeed, the disclosed method will find particular application at the commercial scale, where it can be employed to efficiently refold large quantities of protein.

The present disclosure will now be illustrated by reference to the following examples, which set forth certain embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

The Examples presented herein demonstrate that thiol-pair ratio and redox buffer strength is a significant consideration in achieving an efficient refolding reaction that is insensitive to environmental influences and aeration. This insensitivity is a consideration for the ease of scaling and on an industrial or commercial scale, the transfer of the process from plant to plant.

The Examples also demonstrate that at typical refolding reaction concentrations (0.01-2.0 g/L); the sensitivity to external aeration is relatively muted. However, at concentrations of about 2 g/L and above, the sensitivity of the refold reaction to the thiol-pair ratio and redox buffer strength is increased and nearly all of the chemical components, especially the redox components, may need to be adjusted to accommodate for changes in the protein concentration in the reaction.

Example 1

Expression of Recombinant Protein

In one experiment, recombinant proteins comprising an Fc moiety were expressed in a non-mammalian expression system, namely *E coli*, and driven to form cytoplasmic deposits in the form of inclusion bodies. For each protein refolded the following procedure was followed.

After the completion of the expression phase, the cell broth was centrifuged and the liquid fraction removed, leaving the cells as a paste. The cells were resuspended in water to approximately 60% of the original volume. The cells were then lysed by means of three passes through a high pressure homogenizer. After the cells were lysed, the lysate was centrifuged in a disc-stack centrifuge to collect the protein in the solid fraction, which was expressed in a limited solubility non-native form, namely as inclusion bodies. The protein slurry was washed multiple times by repeatedly resuspending the captured solids slurry in water to between 50% and 80% of the original fermentation broth volume, mixing, and centrifugation to collect the protein in the solid fraction. The final washed inclusion bodies were captured and stored frozen.

Example 2

Identification of Refold Conditions/Redox Components

Multiple complex, microbial-derived proteins were evaluated. Each protein was solubilized in an appropriate level of guanidine and/or urea, typically at levels the equivalent of 4-6 M guanidine or 4-9 M urea, or combinations of both denaturants, which fully denatured the protein. The protein was reduced with DTT, 5-20 mM, at pH 8.5, and incubated at room temperature for approximately 1 hour.

Identification of the refold buffer was performed for each protein. A multifactorial matrix or a series of multifactorial matrices were evaluated to identify the refolding reaction for conditions that optimize yield and minimize aggregate formation. An identification screen was set up to systematically evaluate urea, arginine, glycerol and pH in a full factorial matrix, with each component varied over a range of at least three concentration or pH levels with all other parameters kept constant. The completed reactions were evaluated by RP-HPLC and SE-HPLC analysis for yield and product quality using standard multivariate statistical tools. A subset of the conditions having the desired behavior was then further evaluated in subsequent screens that evaluated a range of pH, thiol-pair ratio, thiol-pair buffer strength, and potentially further excipient levels in a factorial screen. Secondary interactions were also evaluated using standard multivariate statistical tools.

Best results, as determined by reversed-phase and size exclusion HPLC analysis, were observed using a refold buffer containing a denaturant (e.g., urea, dimethyl urea or other chaotrope at non-denaturing levels at levels between 1 and 4 M), an aggregation suppressor (e.g., arginine at levels between 5 and 500 mM), a protein stabilizer (e.g., glycerol or sucrose at levels between 5 and 40% w/v) and a redox component (e.g., cysteine or cystamine). The thiol-pair ratio and redox buffer strength were determined using an experimental matrix of thiol-pair ratio (0.1 to 100, more typically 1 to 25) versus buffer strength (typically 2 mM to 20 mM, depending on the protein concentration, the number of cysteine residues in the protein, and the concentration of reductant used to solubilize the inclusion bodies).

Individual reactions were formed with varying levels of cysteine and cystamine that would allow for a controlled matrix of thiol-pair ratio at various thiol-pair buffer strengths. The relationships were calculated using Equations 3 and 4. Each condition was screened under both aerobic and non-aerobic conditions, utilizing the techniques described herein. Optimum conditions were selected to meet a stable balance of yield, desired distribution of folding species, insensitivity to environmental oxidants (e.g., air), and insensitivity to normal variation in DTT carry-over from the solubilization step.

Example 3

High Concentration Refolding of Non-Native Soluble Protein Form Captured from Cell Lysate

In one experiment, a recombinant protein comprising a plurality of polypeptides joined to an Fc moiety was expressed in *E. coli* as an intracellular soluble peptide chain, lysed from harvested and washed cells, isolated from the lysate by affinity chromatography, and then refolded at a concentration of approximately 12 g/L, as described herein.

After the completion of the expression phase, an aliquot of whole fermentation broth was centrifuged and the liquid fraction removed, leaving the cells as a paste. The cells were resuspended in water to approximately 60% of the original volume. The cells were then lysed by means of three passes through a high pressure homogenizer. After the cells were lysed, the lysate pool was mixed in the presence of air for 8-72 hours to allow for dimerization of the peptide chains. Following the dimerization process, the peptide chain of interest was

isolated from the lysate pool using a Protein A affinity chromatography column. The Protein A column elution pool was mixed at a ratio of 8 parts Protein A elution material to 2 parts of a refold buffer containing urea (10 M), arginine-HCl (2.5 M), Tris at pH 8.5 (1050 mM), and cysteine (10 mM, 5 mM, or 4 mM) and cystamine (4 mM). The diluted mixture was titrated to pH 8.5 and incubated at approximately 5° C. under nitrogen until a stable pool was achieved (~24 hours.) Yields of desired product of approximately 30-80% were obtained a depending on the redox condition evaluated.

In order to emulate the non-anaerobic conditions similar to those typically present in very large-scale protein production processes several steps were taken. When reaction volumes were less than approximately 15 L the refold vessel headspace was purged with nitrogen to limit the effect oxygen could have in the system. The vessel was then sealed and incubation began.

When reaction volumes were more than approximately 15 L but less than 500 L, the refold buffer was prepared and allowed to equilibrate at approximately 5° C. to achieve a stable oxygen level in the solution (typically 50% to 70% dissolved oxygen, relative to air saturation). Once the refold mixture was formed, the vessel headspace was purged with nitrogen to limit any additional effect oxygen could have in the system, the vessel was sealed and incubation period initiated.

Example 4

High Concentration Refolding from Inclusion Bodies

In one experiment, a recombinant protein comprising a biologically active peptide linked to the C-terminus of the Fc moiety of an IgG1 molecule via a linker and having a molecular weight of about 57 kDa and comprising 8 disulfide bonds, was expressed in *E. coli* as inclusion bodies, harvested, washed, concentrated, solubilized, and refolded at a concentration of 6 g/L as described herein.

An aliquot of frozen concentrated inclusion bodies were thawed to room temperature and mixed with an appropriate amount of guanidine and/or urea to generate a denaturant level equivalent to 4-6 M guanidine, which fully denatures the protein. The protein was then reduced with DTT, at 5-20 mM, at pH 8.5, and incubated at room temperature for approximately 1 hour. After the inclusion bodies were dissolved, denatured and reduced, they were diluted into a refold buffer containing urea (1-5 M), arginine-HCl (5-500 mM), glycerol (10-30% w/v), and the identified levels of cysteine and cystamine as determined by the procedure described in Example 2. The final component concentrations are 4 M urea, 150 mM arginine HCl, 20.9% (w/v) glycerol, 2.03 mM cysteine, and 2.75 mM cystamine. The level of dilution was chosen to balance the dilution of the denaturants from the solubilization, maintain the thermodynamic stability of the molecule during refolding, and maintain the highest possible protein concentration in the refold mixture. The diluted mixture was titrated to an alkaline pH (between pH 8 and pH 10) and incubated at 5° C. under non-aerobic conditions until a stable pool was achieved (12-72 hours), as determined by relevant analytical measurements. The resulting process was demonstrated to show stable scalablity from 1 L-scale to 2000 L-scale (see FIG. **3**). Yields of desired product of approximately 27-35% were obtained at both scales. The distribution of product related impurities was also maintained within a tight variance (see FIG. **3**).

Oxygen mass transfer at small-scale is readily achieved and should be inhibited in order to emulate the relatively poorer mass transfer observed at large-scale, where the volume of refold solution is large relative to the volume of air and surface area present at the surface of a large-scale vessel. Thus, in order to emulate the non-anaerobic conditions similar to those typically present in very large-scale protein production processes several steps were taken. When reaction volumes were less than approximately 15 L the refold buffer was sparged with nitrogen to strip oxygen from the solution, the components were dispensed under a blanket of nitrogen and once the refold mixture was formed, the vessel headspace was purged with nitrogen to limit the effect oxygen could have in the system. The vessel was then sealed and incubation began.

When reaction volumes were more than approximately 15 L but less than 500L, the refold buffer was prepared and allowed to equilibrate at approximately 5° C. to achieve a stable oxygen level in the solution (typically 50% to 70% dissolved oxygen, relative to air saturation). Once the refold mixture was formed, the vessel headspace was purged with nitrogen to limit any addition effect oxygen could have in the system, the vessel was sealed and the incubation period was initiated.

At scales greater than 500 L the refold buffer was prepared and allowed to equilibrate at approximately 5° C. to achieve a stable oxygen level in the solution (typically 50% to 70% dissolved oxygen, relative to air saturation). Once the refold mixture was formed, the vessel was sealed and the incubation period was initiated.

The protein concentration of the refold mixture was 6 g/L, which is a four-fold enhancement over the recovery of 1.5 g/L obtained using a method other than the method described in this Example. Overall annual process productivity, in one specific manufacturing facility, was calculated to be increased by >930% due to increased volumetric efficiency in the existing facility tanks.

Example 5

Effect of Thiol-Pair Oxidation State on Disulfide Pairings

FIGS. **1***a*-**1***f* demonstrate that as the thiol-pair ratio is forced to a more oxidizing state (lower thiol-pair ratio), a higher proportion of product species have oxidized amino acid residues and mixed disulfide forms. As the thiol-pair ratio is driven to a more reductive state (higher thiol-pair ratio), this results in lower levels of oxidized amino acid variant species and higher levels of product species with incorrect disulfide pairings or unformed disulfide bonds. As the overall thiol-pair buffer strength is modified, the corresponding optimal thiol-pair ratio is shifted. This effect is similar to how buffer strength modulates the sensitivity of pH to acid and base additions in a buffered solution.

An optimal balance of species was attainable. As shown in FIGS. **1***a*-**1***f*, there is a clear relationship between thiol-pair buffer strength and thiol-pair ratio that can be identified to maintain the optimal species balance and thus facilitate efficient refolding of low solubility proteins. The ability to control product variant species, such as incorrectly disulfide-bonded species and misfolded species, via modulation of the thiol-pair ratio and thiol-pair buffer strength, enables efficient, effective and reliable subsequent purification processes.

Example 6

Effect of Non-Aerobic Conditions on Refolding Efficiency

FIGS. **2** and **3** demonstrate that when the thiol-pair buffer strength is selected appropriately, taking into account the protein concentration and number of cysteine residues in the protein, the sensitivity to external influences, such as oxygen, is significantly reduced. This allows for a non-aerobic refolding condition that is significantly easier to transfer between scales and reactor configurations.

FIG. **2** compares the RP-HPLC analytical species distribution between a 15 L-scale refold and a 20 mL-scale refold under several environmental conditions. For Condition 1 (the trace labeled "1" in FIG. **1**), the solubilization chemicals and solutions were dispensed in air and the refold mixture was incubated in air. In Condition 2 solubilization chemicals and solutions were dispensed in air and incubated under nitrogen headspace. In Conditions 3-7 solubilization chemicals and solutions were dispensed under nitrogen overlay conditions and in conditions 3, 5, 6, and 7 solubilization chemicals and solutions were incubated under nitrogen. In Condition 7, the refold solution was also stripped of nitrogen prior to combination with the solubilization solution. In Condition 4 the solubilization chemicals and solutions were incubated under ambient air conditions.

The results shown in FIG. **2** demonstrate that the conditions under which the solubilization chemicals and solutions were dispensed or incubated in the presence of air (i.e., Conditions 1, 2, and 4) do not achieve results that are comparable to the larger-scale control. In Conditions 1, 2 and 4, increased formation of oxidized species (pre-peaks) are observed. The pre-peaks are indicated by arrows in the panels for Conditions 1, 2 and 4.

FIG. **3** compares the RP-HPLC analytical results of an identified condition, achieved as described in Example 2, at 1 L-scale and 2000 L-scale. In this figure, essentially no difference in the distribution of species is detectable. Taken together, FIGS. **2** and **3** demonstrate that when aeration is carefully controlled, the small-scale refold reactions are more predictive of those expected upon scale-up of the refold reaction, facilitating the implementation of large-scale protein refolding processes.

What is claimed is:

1. A method of refolding a protein expressed in a non-mammalian expression system and present in a volume at a concentration of 2.0 g/L or greater comprising:

(a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 0.001 to 100 and a redox buffer strength of 2 mM or greater and one or more of:

(i) a denaturant;

(ii) an aggregation suppressor; and

(iii) a protein stabilizer;

to form a refold mixture;

(b) incubating the refold mixture; and

(c) isolating the protein from the refold mixture.

2. The method of claim **1**, wherein the final thiol-pair ratio is selected from the group consisting of 0.05 to 50, 0.1 to 50, 0.25 to 50, 0.5 to 50, 0.75 to 40, 1.0 to 50 and 1.5 to 50, 2 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 30 to 50 or 40 to 50.

3. The method of claim **1**, wherein the thiol-pair buffer strength is selected from the group consisting of greater than or equal to 2.25 mM, 2.5 mM, 2.75 mM, 3 mM, 5 mM, 7.5 mM, 10 mM and 15 mM.

4. The method of claim **1**, wherein the protein is present in the volume in a non-native limited solubility form.

5. The method of claim **4**, wherein the non-native limited solubility form is an inclusion body.

6. The method of claim **1**, wherein the protein is present in the volume in a soluble form.

7. The method of claim **1**, wherein the protein is recombinant.

8. The method of claim **1**, wherein the protein is an endogenous protein.

9. The method of claim **1**, wherein the protein is an antibody.

10. The method of claim **1**, wherein the protein is a complex protein.

11. The method of claim **1**, wherein the protein is a multimeric protein.

12. The method of claim **1**, wherein the protein is an Fc-protein conjugate.

13. The method of claim **1**, wherein the non-mammalian expression system is one of a bacterial expression system and a yeast expression system.

14. The method of claim **1**, wherein the denaturant is selected from the group consisting of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea.

15. The method of claim **1**, wherein the protein stabilizer is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

16. The method of claim **1**, wherein the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

17. The method of claim **1**, wherein the thiol-pairs comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

18. The method of claim **1**, wherein the incubation is performed under non-aerobic conditions.

19. The method of claim **1**, wherein the isolation comprises contacting the mixture with an affinity separation matrix.

20. The method of claim **19**, wherein the affinity separation matrix is a Protein A resin.

21. The method of claim **19**, wherein the affinity resin is a mixed mode separation matrix.

22. The method of claim **1**, wherein the isolating comprises contacting the mixture with an ion exchange separation matrix.

23. The method of claim **1**, wherein the isolating further comprises a filtration step.

24. The method of claim **23**, wherein the filtration step comprises depth filtration.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that on this 5th of December, 2016, I caused the foregoing BRIEF OF PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING LIMITED to be filed with the Clerk of the Court using the CM/ECF system. I also caused a true and correct copy of the foregoing BRIEF OF PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING LIMITED to be electronically served on Defendants-Appellees Apotex Inc. and Apotex Corp.'s counsel of record, as follows:

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CERTIFICATE OF COMPLIANCE

This brief complies with the type-volume limitation of Fed. R. App. P. 32(a)(7)(B). The brief contains 13,718 words, excluding parts of the brief exempted by Fed. R. App. P. 32(a)(7)(B)(iii) and Federal Circuit Rule 32(b). The word count includes the words counted by the Microsoft Word 2010 function. This brief also complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6). The brief has been prepared in a proportionally spaced typeface using Microsoft Word 2010 in 14-point font of Times New Roman.

Dated: December 5, 2016

/s/ Nicholas Groombridge
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